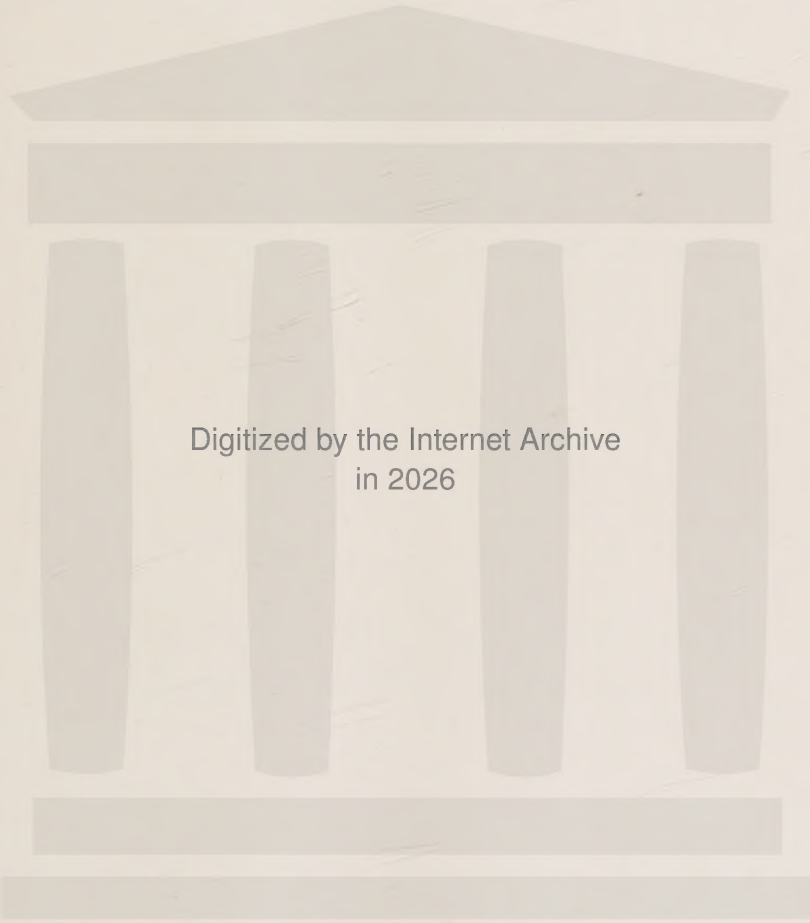


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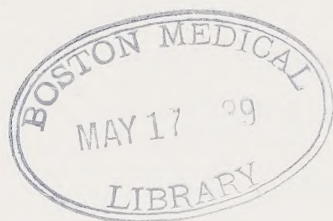
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THE EFFECT OF SYNTHETIC MALE HORMONE SUBSTANCE UPON FOLLICULAR GROWTH AND OVULATION IN THE GUINEA PIG¹

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ONE PLATE (FIVE FIGURES)

Male hormone substance has been shown to inhibit the reproductive cycle of the female animal (Robson, '36; Zuckerman, '37) and woman (Hamilton and Allen, '38). In view of the clinical use of androgenic material in conditions of menorrhagia, metrorrhagia and phenomena that may accompany the menopause, an attempt was made to study in a quantitative manner its suppression of ovulation. The extensive data (see Young, '37, for references) which have been previously collected regarding follicular growth, ovulation and behavior in the guinea pig rendered this animal peculiarly suitable.

MATERIAL AND METHODS

Twenty-three adult female guinea pigs were observed prior to experimentation to assure selection of only those which had a normal length of cycle and behavioral responses typical of heat and ovulation (Young, Dempsey and Myers, '35). The animals chosen had a cycle length of 16 ± 1 days for one or more cycles and were observed to exhibit lordosis and characteristic heat responses for at least two periods of heat.

Injections were dated from the end of that period of heat, which was considered as the first day of the cycle. A standard

¹Aided by the International Cancer Research Foundation and by a grant to Prof. William Young from the Committee for Research on Problems of Sex, National Research Council.

daily dose of 4 mg. of testosterone propionate² in 0.2 cc. of peanut oil was administered subcutaneously. Nineteen of the guinea pigs were separated into four groups injected respectively beginning the first, fifth, ninth or thirteenth day of the cycle; the purpose of this was to test for any possible influence due to the time of the cycle at which injections were begun. In each group some of the animals were sacrificed the day following the last injection for study of the inhibition of follicular growth. In these animals injections were continued through at least the eighteenth day of the cycle so that the normal time of heat recurrence was always included in the period of injection (table 1). Other animals of each group were observed to determine the delay between the cessation of injections and the return of heat and the normality and fertility of reproductive cycles following cessation of androgen administration. The final injection in these later cases fell on the eighteenth to thirtieth day following the heat from which the injections were dated.

A further series of twelve guinea pigs received injections of 0.5 to 0.2 mg. of testosterone propionate for 5 months, followed by implantation of 20 mg. of testosterone propionate crystals monthly for the succeeding 4 months. A continued level of androgenic substance was maintained over a period of 9 months.

Nine and one-half hours before autopsy each animal was injected subcutaneously with an aqueous solution of colchicine, 75 γ per 100 gm. of body weight. The ovaries were fixed in Bouin's fluid, sectioned serially at 10 μ and stained with hematoxylin and eosin.

Two normal females killed 9½ hours after heat and two others killed on the fifth day of the cycle were used for study of the effect of colchicine alone. The ovaries of over 100 normal guinea pigs were already available for comparison.

The follicular volumes were estimated by the method previously described by Myers, Young and Dempsey ('36), in which

² Testosterone propionate was furnished through the courtesy of the Ciba Company under the trade name Perandren.

TABLE 1

Daily injections of 4.0 mg. of testosterone propionate prevented ovulation in the guinea pig as shown by the absence of vaginal opening or heat and by the suppression of follicular growth, so that the volume of the follicles never exceeded that observed on the fifth to the eighth day of reproductive cycle in the normal animal. Shortly after the end of injections cyclic reproductive function returned and the animals proved to be fertile

ANIMAL	DAY OF CYCLE INJECTIONS BEGUN	NUMBER OF INJECTIONS	DAYS BETWEEN FINAL INJECTION AND			FOLLICULAR VOLUMES						CORPORA LUTEA		REMARKS
			Autopsy	Vaginal opening	Heat	Four largest active follicles		Four largest atretic follicles						
						Average ($\times 10^6 \mu^3$)	Range ($\times 10^6 \mu^3$)	Average ($\times 10^6 \mu^3$)	Range ($\times 10^6 \mu^3$)	New	Old			
72	1	18	1			43.6	39.4-49.1	51.5	36.2-63.5	0	3	Old corpora in last stages of atresia Only 1 ovary available Pregnant Not sacrificed		
77	1	18	1			95.3	92.5-104	66.5	53.9-93.5	0	3			
51	1	18	11	10	11					4	3			
73	1	18	10	1	10					1	1			
80	1	18		12	12									
68	1	18		12	12									
78	5	14	1			127	108-155	126	118-137	0	3	Not sacrificed Not sacrificed Pregnant; bore and reared two young		
86	5	17	1			50.0	44.2-58.1	42.2	31.5-64.5	0	3			
57	5	17		10										
65	5	17	11	8	11					3	2			
70	9	10	1			82.3	67.0-104	82.3	63.0-94.5	0	2	Old corpora in last stages of atresia Pregnant; bore three healthy young		
76	9	10	1			41.3	34.2-59.5	47.2	33.5-56.5	0	2			
52	9	17		13										
67	9	17		11										
92	9	17		12										
74	13	6	1			58.2	50.1-66.5	91.1	60.4-160	0	2	Sacrificed on fifth day Sacrificed on fifth day Sacrificed 9½ hours after heat Sacrificed 9½ hours after heat		
79	13	6	1			59.0	50.7-67.8	65.0	57.5-74.8	0	3			
53	13	17	16	11	16					3	3			
75	13	17		11										
88	Control					22.8	14.9-26.2			3	3			
101	Control					56.8	48.7-64.2			2	1			
63	Control									3	3			
102	Control									2	2			

which measurements were taken of the diameter of the follicle within the membrana granulosa. In each follicle the two largest diameters at right angles to each other were measured with a micrometer eye-piece. The third diameter was obtained by counting the number of sections through which a follicle extended and multiplying this figure by the thickness of the sections. "Since the follicles were not perfect spheres, a modification of the formula $\frac{4}{3} r^3$ for determining spherical volumes was used, the product of the radii of the 3 dimensions being substituted for r^3 . The volumes are expressed as cubic micra." Determinations of the average follicular volume of each ovary were made by taking an average of four of the largest active follicles and four of the largest atretic follicles in each ovary. The range in volume was also examined.

As an example of determinations by this method, the average volume of the four largest follicles in both ovaries of no. 79 is as follows: Diameters of the follicles in figure 1 are 490μ , 492μ and 400μ . The radii of these, substituted in the formula $\frac{4}{3} r^3$, give a volume of $50.7 \times 10^6 \mu^3$. The volumes of the other three follicles are $54.8 \times 10^6 \mu^3$, $67.8 \times 10^6 \mu^3$ and $62.7 \times 10^6 \mu^3$, the average of the four being $59.0 \times 10^6 \mu^3$ (table 1).

RESULTS

Male hormone substance prevented ovulation as shown by 1) the lack of behavioral responses characteristic of the period of heat and ovulation, 2) the failure of the vagina to open and 3) after the cessation of androgenic treatments, the return of cyclic vaginal opening, heat, receptivity to the male, with ensuing pregnancy and the rearing of offspring. Moreover, follicular growth as well as ovulation was inhibited as seen. the appearance of the ovaries upon operative inspection and upon microscopical examination.

1. *Lack of behavioral responses characteristic of the period of heat and ovulation.* Normally, guinea pigs which come into heat will ovulate, the relationship between behavior and ovulation being even more direct than that between vaginal

smears and ovulation (Young, '37). Behavior is so distinct as to be reliable and easily detected. It consists of the following responses to touching of the genital area or stroking of the back: a) lordosis, the lower portion of the back being distinctly bowed, b) a wide stance of the hind legs which are extended laterally and caudally; locomotion is difficult as long as this posture is maintained, c) elevation of the hind quarters of the guinea pig and a spreading apart of the vulva; this causes the perineum to project backward so as to 'present' to the male, d) emission of a purring sound and the elevation of body hair in some regions. These responses may be followed by e) acceptance of the male.

These behavioral responses remained absent throughout the course of treatment with testosterone propionate and for some days following the cessation of treatment (table 1). It should be noted that the periods of injection and inspection of each animal were selected so as to include at least one expected heat period.

2. *Failure of the vagina to open.* In normal, untreated animals the vagina opens periodically just before estrus and remains open for a day or more after the disappearance of heat. Occasionally a guinea pig will come into heat and manifest true heat behavior before the vagina opens (Young, Dempsey and Myers, '35) but invariably the vagina opens during the period of estrus. Since observations of the animals during the present experiments covered only 18 hours of the day, from 7.00 A.M. to 1.00 A.M. the absence of vaginal opening during and following injections was perhaps even more conclusive proof, than the lack of heat, that ovarian growth had not culminated in ovulation and estrus. The period of heat is short according to Young, Dempsey and Myers ('35), averaging about 8.2 hours and lasting only 1 hour in some cases; since heat occurs more commonly in early morning hours, the incidence of heat might have been overlooked in some instances due to the lack of 24-hour observations but the long period in which the vagina remains open could not have escaped attention. These periods during which

the vagina remained closed included in all cases the time when the vagina would have been expected to open, if androgenic material had not suppressed the ovarian cycle.

3. *After the cessation of androgenic treatment cyclic opening of the vagina, heat, and receptivity to the male returned, followed by pregnancy and the rearing of offspring.* Table 1 shows that the vagina opened 8 to 13 days following the cessation of injections in ten animals. Another animal (no. 73) had vaginal opening the day after the last injection but smears of the vagina showed only superficial cells and leucocytes; since heat did not ensue for 11 days, the vagina may have been forced open. The time of vaginal opening would then be in agreement with that of the rest of the animals in being near the period of heat (table 1). Heat itself was observed in six of the animals 10 to 16 days after the end of injections. One animal (no. 80) which was observed in heat, and two (nos. 75 and 92) in which heat was not detected possibly because of its occurrence during hours when the investigators were not present, were mated and bore viable litters—proof of a post-treatment/return of ovulation and fertility. Guinea pig no. 80 successfully reared three young; no. 92 gave birth to and raised two young. Three animals born to no. 75 were sacrificed for use in another study.

4. *Follicular growth as well as ovulation was inhibited, as seen by the appearance of the ovaries upon operative inspection and microscopical examination.* Ovaries removed on the day following the final injection of testosterone propionate were smaller than the ovaries of control animals killed on the first and fifth days of the cycle or those of experimental animals not sacrificed after the end of injections until heat occurred. The small size of the ovaries was more pronounced in the animals which received the greatest number of daily injections. The larger size of the ovaries of the animals which received fewer injections, especially animals no. 74 and no. 79, appeared to be due to the presence of many atretic remnants of the large follicles present at the beginning of the period of injections. When injections are continued for

a longer period these had sufficient time to disappear. In the case of animals in which injections were begun on the fifth and ninth days no large follicles were present at the beginning of the injection period. The uteri and vaginae were also smaller and less well vascularized in the animals injected for the longer periods.

No ovulation points were present, a fact in agreement with the small size of the follicles and the lack of any new corpora lutea. The size of the follicles was uniformly small, the volumes of the largest follicles being comparable to those found from the fifth to the eighth day of the cycle in normal animals.

The volumes of the follicles of both ovaries of animals killed the day after the final injection were compared a) with regard to influence of the time of the cycle when injections were begun (Wolfe and Hamilton, '37 a), b) with those of guinea pigs allowed to recover from injections and enter a period of heat before autopsy and c) with those of control animals which received no injections. In the animals sacrificed on the eighteenth day of the cycle and while under the influence of testosterone propionate there was no significant difference in the size of active follicles whether the injections were begun on the first, fifth, ninth, or thirteenth day of the cycle, even though the animals received 18, 14, 10 or 6 injections respectively. The volumes of the four largest active follicles of guinea pigs injected beginning the first day of the cycle averaged $43.6 \times 10^6 \mu^3$ in pig no. 72 and $95.3 \times 10^6 \mu^3$ in no. 77. When the injections were begun on the fifth day of the cycle the average follicular volume was $127 \times 10^6 \mu^3$ in no. 78 and $50 \times 10^6 \mu^3$ in no. 86. With the start of injections on the ninth day of the cycle the average was $41.3 \times 10^6 \mu^3$ in no. 76 and $82.3 \times 10^6 \mu^3$ in no. 70. When begun the thirteenth day the average was $59 \times 10^6 \mu^3$ in no. 79 and $58.3 \times 10^6 \mu^3$ in no. 74 (fig. 3). Control animals which received colchicine but no hormone and were killed on the fifth day of the cycle had a follicular volume of $22.8 \times 10^6 \mu^3$ in no. 88 and $56.8 \times 10^6 \mu^3$ in no. 101.

The range of the volumes of the four largest active follicles was also alike in animals whose injections were initiated at different stages of the cycle (table 1). These volumes and range of volumes and data obtained by Myers, Young and Dempsey ('36) indicate that the follicular volume was maintained at about that of the guinea pig in the fifth to eighth day of a normal cycle. This holds irrespective of whether follicular development prior to treatment had not reached that stage, as in animals nos. 72, 77, 78 and 86, or had already progressed beyond that stage as in animals no. 76, 70, 79 and 74. It appears that the later stages of follicular growth, maturation and ovulation were suppressed, but not the early stages, testosterone propionate in the dose given allowing the follicles to grow to a size roughly comparable to that of the largest follicles in the fifth to the eighth day of the normal cycle (fig. 1). Follicles larger than this before treatment did not continue to mature and rupture but apparently underwent regression, for whatever the time of the cycle at which injections were begun the animals had the same common denominator in volume of the active follicles.

The occurrence of heat 10 to 12 days after the end of injections in five of the six animals observed in heat is rapid in comparison with the ordinary 16-day interval of follicular growth. Moreover, the time elapsing between the end of the inhibiting influence of the hormone and the appearance of heat and ovulation is probably shorter than 10 to 12 days, for the cessation of injections does not immediately end the effectiveness of the administered hormone. Testosterone propionate in peanut oil is slowly absorbed over a period of many days, a single injection of 5 mg. in 0.1 of peanut oil exerting a detectable effect for 17 days in the chick (Hamilton and Dorfman, '38). It has been shown that follicles will mature in an average of 11 days and ovulate at a less than normal ovulation-size if corpora lutea are excised at the time of heat (Loeb, '11; Dempsey, '37).

The volumes and range of volumes of the atretic follicles (fig. 2) were also roughly alike whether the injections were

begun early or late in the cycle (table 1): no very large atretic follicles were present, but some of the largest of the follicles were in an atretic condition. There was a greater number of atretic follicles in the ovaries of animals under the influence of the hormone than in uninjected animals at the fifth to eighth day of the cycle. Follicles were found in various stages of atresia from the breaking down of the membrana granulosa to an infiltration with connective tissue elements. Atresia of the ova was also present in different stages, pycnotic and eccentric nuclei occurring in some cases, while multipolar spindles and fragmenting ova were frequently encountered.

Noteworthy too was the fact that ovulation continued absent over long as well as short periods of injection. The follicles were of the same size whether the guinea pigs were injected for 6 or 18 days before autopsy. Further, those guinea pigs treated for 9 months with androgens apparently did not ovulate during this time, since vaginal opening and heat were not observed. Sexually active males were kept in the same cages with the injected females during this time but pregnancy did not occur. The males were fertile as proven by matings with non-injected females which were placed in the cages at intervals.

After 9 months of treatment injections were stopped in two guinea pigs and vaginal cycles appeared within 3 weeks. The occurrence of the reproductive cycle was all the more interesting since the animals had received injections beginning shortly after birth. This early return of cyclic reproductive function after the end of treatment is in agreement with the findings in chickens that after daily treatment of chicks with large amounts of testosterone propionate (0.5 mg. in 0.1 cc. of peanut oil) for over 5 weeks, injected animals were less than 5 weeks later than control animals in beginning egg laying (Hamilton, '38). Each of these two guinea pigs were mated after the end of the long periods of injections and bore and reared 1 young.

The lack of ovulation while the animals were on treatment was corroborated by the absence of new corpora lutea in all animals killed the day after the close of injections (table 1 and fig. 5). Old corpora lutea remained from pre-injection ovulation but new corpora occurred only after ovulation following cessation of treatment (fig. 4). The old corpora in animals killed at the end of the first heat after the close of the injection period were barely discernible due to the advanced state of atresia.

COMMENT

The above data show that sufficiently high doses of testosterone propionate (4 mg. daily) will suppress follicular growth and ovulation in the guinea pig. Using the same kind of animals but one-fortieth of this dose, Dempsey ('37) found that 0.1 mg. daily did not prevent ovulation. Duncan, et al. ('38) state that inhibition of the sexual cycle with testis tissue extracts is due to phospholipin impurities in the extract; but inhibition of the reproductive cycle has been produced with synthetic androgenic substances in rodents, monkeys and human.

The use of testosterone propionate in women, as in treatment of menorrhagia and metrorrhagia must be considered from the standpoint of coincident suppression of ovulation. Other influences to be considered in administration of this material to females are: 1) masculinization (Hamilton, '37) and 2) the production of pseudo-hermaphroditic forms (Dantchakoff, '37; Greene and Ivy, '37; Hamilton and Gardner, '37; Hamilton and Wolfe, '38 b).

The suppression of ovulation by androgens is held most likely to be due to an action upon the pituitary. Studies with transplantation experiments in rats have shown that the gonadotropic power of the pituitary is markedly reduced (Hamilton and Wolfe, '38 a). Pituitaries from uninjected castrated rats stimulated growth of test animal ovaries to an average of 65 mg., whereas pituitaries from injected castrate litter mates who received testosterone propionate produced

ovaries averaging only 23.3 mg. Marked histological changes are also observed in the pituitary after treatment of the animal with androgens (Wolfe and Hamilton, '37 a, b, c).

SUMMARY

1. Adequate administration of synthetic male hormone substance, testosterone propionate (4 mg. daily in 0.2 cc. peanut oil), suppressed follicular growth and ovulation in nineteen guinea pigs during a 6- to 18-day period of injection. Ovulation was prevented as shown by 1) the lack of behavioral responses characteristic of guinea pig periods of heat and ovulation, 2) the failure of the vagina to open, 3) the atrophic condition of the ovaries as seen upon operative inspection, and 4) microscopical examination of the ovaries which showed a) average volume and range of the largest active follicles about that of untreated guinea pigs on the fifth to eighth day of a 16-day cycle, b) a large number of atretic follicles and c) no new corpora lutea.

2. In twelve other females the continued administration of testosterone propionate prevented ovulation during 9 months of injection.

3. The reproductive cycle and ovulation returned soon after the cessation of androgen injections; the vagina opened 8 to 13 days after the end of six to eighteen injections in ten of eleven animals observed thereafter. Some of these guinea pigs were found to be in a period of heat 6 to 10 days after the opening of the vagina and two others were mated with subsequent bearing and rearing of normal offspring. Two more guinea pigs injected continuously for 9 months bore and raised young when injections were stopped.

4. Ovulation occurred in a shorter time after the cessation of injections than it does in a normal animal following a period of heat.

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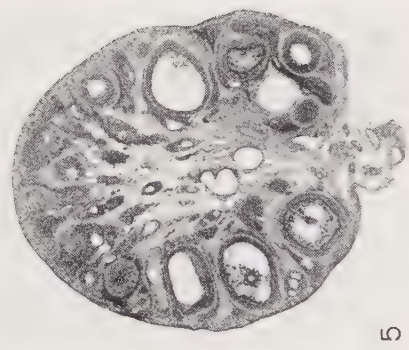
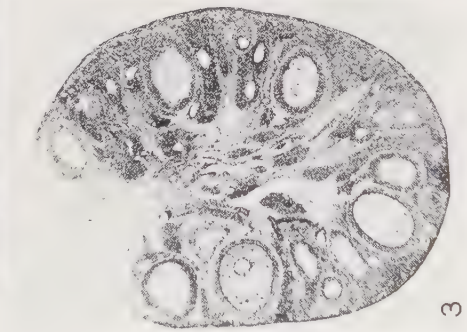
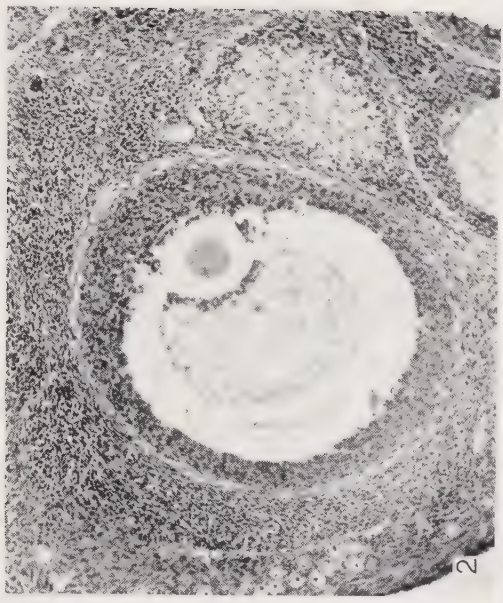
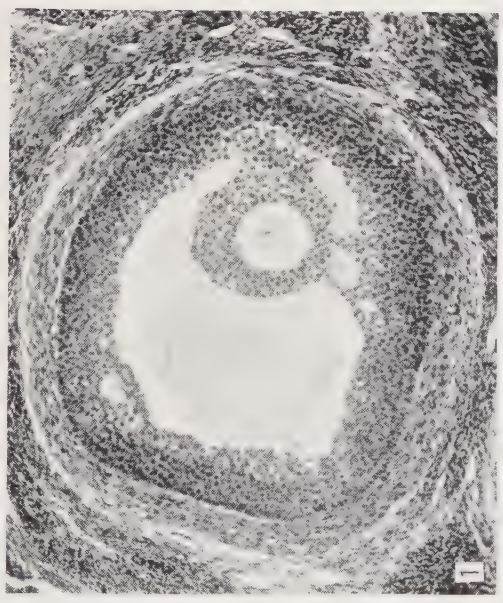
PLATE I

EXPLANATION OF FIGURES

In animals receiving injections of 4 mg. of testosterone propionate daily, the ovaries are reduced in size and the volume of even the largest active follicles is small. The large active follicle pictured in figure 3 has a volume of $50.7 \times 10^6 \mu^3$ and is from guinea pig no. 79. $\times 25$.

The follicles grow only to the stage found in normal animals in the fifth to eighth of the cycle and then degenerate. Figure 1 is of a follicle of average size from the ovary of an untreated animal on the fifth day following heat. Figure 2 is of a large atretic follicle from the ovary of an adrogen-injected animal, no. 70, showing the low membrana granulosa, an absence of mitosis in the theca and a multipolar spindle in the ovum. The volume of the follicle is $63 \times 10^6 \mu^3$. $\times 125$.

Figure 5 of animal no. 86, which was sacrificed while the androgenic injections were still effective, and figure 4 of animal no. 65, which lived after injections until heat and ovulation returned, may be contrasted as follows: after the end of treatment the ovary regains a large size with mature ovulating follicles which are succeeded by corpora lutea. $\times 25$.



RELATIVE VISCOSITIES OF TUMOR CELLS AS DETERMINED BY THE ULTRACENTRIFUGE¹

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TWO PLATES (NINETEEN FIGURES)

In testing various normal and malignant tissues for resistance to displacement of cellular contents when centrifuged at extremely high speeds it was found that tumor cells, particularly those of adenofibromas and carcinomas, showed little or no derangement of contents while normal cells from such structures as adrenal cortex or anterior pituitary gland commonly became completely stratified with nucleus and various of the heavier cytoplasmic inclusions forced to one side of the cell while the Golgi apparatus came to occupy the opposite side (Guyer and Claus, '36 a, b; Dornfeld, '36). The present paper is an extension of our earlier study. In general, in this experiment, in the four types of tumors studied with the ultracentrifuge, the order of resistance to displacement of cellular contents was 1) rat adenofibroma, 2) rat carcinoma, 3) mouse adenocarcinoma and 4) rat sarcoma.

In centrifuging, a small bit of the tissue to be tested was placed in isotonic Locke's solution in the rotor of a Beam's air-driven ultracentrifuge and rotated at a speed which produced a displacement pull of about 400,000 times that of gravity.

In order to get a satisfactory check with normal cells, in the case of the rat carcinoma, bits of the cancer (Flexner-Jobling carcinoma) to be tested were implanted in such

¹ This investigation was supported by research grants from The Jonathan Bowman Memorial Fund and The Brittingham Trust Fund.

normal tissues as adrenal, kidney, pancreas, liver, spleen, stomach and intestine, in some twenty animals. The carcinoma grew actively in all the tissues to which it was transplanted except the spleen. In spleen it seemed to grow with difficulty or even in certain cases to be actively repelled. When the malignant growth was well established in host tissues small pieces of united host and graft tissue were excised and centrifuged together, thus submitting normal and malignant cells to the same treatment. Invariably the cells of the carcinoma fraction of the centrifuged piece showed little or no displacement of contents while the cells of the associated fraction of normal tissue revealed noticeable and sometimes marked derangement or even stratification of the cellular components. This is well shown in figures 1, 2 and 5, from an adrenal-carcinoma graft. In the cell from the adrenal cortex (fig. 1) the nucleus has been forced to one side together with the greater part of the cytoplasm. The centripetal side of the cell is occupied by what appears to be a watery vacuole but which would probably be found to represent the Golgi apparatus, as in figure 5, if similarly stained to reveal this structure. The nuclear inclusions have been thrown toward the centrifugal side with only strands of linin-like material remaining attached to the opposite wall. In contrast to this a carcinoma cell (fig. 2) from the same tissue graft, and therefore centrifuged along with the cell just described, remained wholly undisturbed. The large nucleus with centrally disposed nucleolus still occupies the center of the cell cytoplasm and retains the appearance of carcinoma cells in control uncentrifuged preparations. Figure 3 is a drawing of a section through a carcinoma cell from the same adrenal-carcinoma graft, showing that notwithstanding an hour of severe centrifuging, the many chromosomes of a cell in late prophase of division are displaced little if any and remain well apart as they do in cells of uncentrifuged carcinoma in a similar stage of division. The same condition prevails in centrifuged carcinoma cells of all sizes whether they bear fewer than the normal number of chromosomes or are giant cells with an excess of chromosomes (fig. 16).

Figures 4 and 5 are sections through cells of the adrenal cortex of adrenal-carcinoma grafts, stained to demonstrate the Golgi apparatus. Figure 4 represents the uncentrifuged condition with the blackened Golgi material mostly grouped on one side of the nucleus in the form of independent granules or more or less of a network. Figure 5 shows a similar cortical cell which has been centrifuged for 1 hour. In it the Golgi material has all accumulated at the centripetal side of the cell in what appears to be a mass of fluid. On the other hand, figure 6 is a drawing of a section through an accompanying carcinoma cell which was centrifuged at the same time for 1 hour. The arrangement of the blackened Golgi elements is not noticeably different from that seen in uncentrifuged carcinoma cells.

The cells of rat sarcomas, however, seem not to be quite so resistant to powerful centrifugalization as carcinomas, although they show less derangement than normal tissue cells. Sarcoma tissue viewed histologically after heavy centrifuging often appears to have become highly vacuolated but closer inspection shows that the vacuolar accumulations of fluid are among the cells rather than in their interiors. There seems to be a considerable quantity of liquid in such tissues and centrifuging makes it accumulate in minute droplets in the spaces in intercellular connective tissue. Occasionally, however, a sarcoma cell itself will show obvious effects of the centrifugalization.

Figure 7 represents a section of a rat sarcoma cell which has been centrifuged for 1 hour. It shows but little indication of such violent treatment. The nucleus has not been moved to the side of the cell and the Golgi material, stained black, has not been displaced from its relationship with the nucleus although it seems to have been a bit consolidated and bears evidence of liquid exudate.

Figure 8 is a section through an uncentrifuged cancer cell of the mouse from a type of spontaneous adenocarcinoma of the breast, and figure 9 a similar adenocarcinoma cell which has been centrifuged for 1 hour. It is evident that the severe centrifuging has produced no visible cellular displacements.

The Golgi apparatus is intact and even the nucleolus of the large nucleus has not been perceptibly shifted.

One of the most noticeable cytological effects of centrifuging various types of cancer cells was the frequent appearance of doubled chromosomes, presumably in cells in late prophase or metaphase of division (figs. 10, 12, 13). Although such doubled chromosomes were seen as rare exceptions in sections of uncentrifuged tumors they were so plentiful in the centrifuged ones that as many as five cells displaying this double effect have been counted in a single field of a high powered (1.8 mm. oil immersion) objective. Corresponding cells in control bits of the same tumors show very frequent mitotic figures, but in general the chromosomes appear to be of heavier type and single. The same result followed centrifuging whether the cell were rat carinoma (fig. 10), rat sarcoma (fig. 12) or mouse adenocarcinoma (fig. 13).

That the doubling of chromosomes may also be induced in dividing normal cells is evidenced by figure 11, which represents a section through a cell from the normal mammary gland of the mouse. In this normal cell, however, unlike the cancer cells, the group of dividing chromosomes has been forced to one side of the cell while the other side has acquired a large watery-looking vacuole. Presumably the stiffness of the cytoplasm immediately surrounding the chromosomes is sufficient to prevent their being clumped into one mass.

In such cases of induced doubling by prolonged centrifugalization apparently the metaphase chromosomes complete their process of division but are prevented from diverging to form daughter nuclei as they normally would do. This interpretation is borne out by the further fact that anaphase figures were rare or lacking in sections from centrifuged material but plentiful in corresponding tissue from uncentrifuged tumors. It would seem that either the central cytoplasm of the dividing cell had become so viscous that the chromosomes could not move through it or that the spindle had been destroyed or its development prevented. Possibly both conditions prevail.

It is interesting to note that some of the divided chromosomes have much the appearance of the early tetrads to be seen in mouse spermatocytes (see for example, fig. 13).

From the foregoing observations the protoplasm of cancer cells in general is evidently more viscous than that of ordinary tissue cells. Since the nuclear contents are as little displaced by centrifuging as are the cytoplasmic, there would seem to be a general stiffening up of the entire cell substance. The only other alternative would be the highly improbable supposition that all of the inclusions of the tumor cells have become of the same specific gravity as the general cytoplasmic ground substance.

Such increased viscosity might be an important factor in the irregular distributions and duplications which are so characteristic of cancer cells. If divided chromosomes are wholly or in part impeded in their divergences toward what would normally be the poles of the division figure this might account for the multiple numbers of chromosomes found in many cancer cells along with the more normal-appearing distributions. On the other hand if anything impeded or prevented spindle formation and thus destroyed the distributional mechanism it is obvious that irrespective of cytoplasmic viscosity a multiplicity of chromosomes within the boundaries of one cell would result. As pointed out, this seems to be what happens in the centrifuged cells. The appearance in the latter is much the same as that which often follows the administration of colchicine to either plant or animal tissues. In fact, in cancerous rats to which colchicine has been administered (0.1, 0.2 or 0.3 mg. per 100 gm. of body weight), the authors found after 24 hours not only the number of mitotic figures increased greatly in the tumors but that the chromosomes often showed an appearance of doubling which was indistinguishable from that resulting from centrifugalization. Since it seems to be the concensus of opinion that colchicine induces polyploidy by suppression of spindle formation it may be that the rotational and the colchicine effects are due

to similar disabilities in the achromatic mechanism. Inasmuch as well-developed and even multipolar spindles are common in cancer tissues it is evident, however, that the suppression of spindle formation, if it exists in ordinary tumor growth, is intermittent or only occasional.

As the result of the work of Warburg ('31) and his associates and successors it is now a fairly well-established fact that the normal respiratory metabolism of cancer cells is so seriously upset that the usual carbon dioxide output is largely replaced by lactic acid, but whether this begins before or follows the irregularities in chromosomal distribution seen so frequently in malignant cells is unknown. Were the sequence respiratory upset and lactic acid output first, and irregularity in chromosomal distribution second, then one might infer that the accumulation of lactic acid featured in some way in increasing cell viscosity which tended in turn to interfere in mitoses by impeding the normal migration activities of the chromosomes. On the other hand it is not improbable, of course, that something first goes wrong with the chromosomal complex of the cell and that this in turn leads to respiratory abnormalities. To call such chromosomal aberrances, if they occur, 'mutations,' is obviously only another way of saying, 'I don't know,' since such naming explains nothing in the way of what causes the initial disorder.

SUMMARY

Judging from the lack of displacement of cellular contents in tumor cells subjected to centrifugalization at extremely high speed, the protoplasm of cancer cells, notably that of carcinomas and adenofibromas, has a decidedly greater viscosity than that of normal tissue cells. It is suggested that this viscosity may at times be an important factor in the accumulation of abnormal numbers of chromosomes in one cell. A lack of proper spindle development may be associated with the increased viscosity. Inasmuch as the polyploidy seen in various plant and animal tissues which commonly follows the administration of colchicine is attributable to suppression

of spindle formation in mitosis and the consequent lack of distribution of the divided chromosomes, it seems probable that the division of chromosomes without polar distribution so evident in cells severely centrifuged may likewise be due to the prevention of spindle development.

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PLATE 1

EXPLANATION OF FIGURES

1 Section of an adrenal cortex cell from an adrenal gland bearing a carcinoma implant, centrifuged for 1 hour; the cellular contents are more or less stratified with the heavier contents accompanying the displaced nucleus. About $\times 1600$.

2 Section of a carcinoma cell from implant in adrenal gland referred to in figure 1, centrifuged for 1 hour with cell shown in figure 1; there is no visible displacement of either nuclear or cytoplasmic contents. About $\times 1600$.

3 Early metaphase chromosomes in section of a carcinoma cell centrifuged for 1 hour; there is no perceptible clumping nor displacement of the chromosomes. About $\times 1600$.

4 Golgi preparation in a section of an uncentrifuged adrenal cortex cell; the black areas indicate Golgi material. About $\times 1600$.

5 Golgi preparation in a section of an adrenal cortex cell from an adrenal cortex bearing a carcinoma, centrifuged for 1 hour; the lighter, disrupted Golgi apparatus has been displaced to one side of the cell; the nucleus, to the other. $\times 1600$.

6 Golgi preparation in a section of a carcinoma cell from implant in adrenal gland referred to in figure 5, centrifuged for 1 hour; the Golgi apparatus is unchanged. About $\times 1600$.

7 Golgi preparation in a section of a rat sarcoma cell, centrifuged for 1 hour; the Golgi apparatus is seen to be somewhat condensed when compared with that of a similar cell from the same piece of tissue. About $\times 1600$.

8 Golgi preparation in a section of uncentrifuged mouse adenocarcinoma cell. About $\times 1600$.

9 Golgi preparation in a section of a mouse adenocarcinoma cell, centrifuged for 1 hour; no perceptible displacement has occurred. About $\times 1600$.

10 Section of a rat carcinoma cell centrifuged for 1 hour; the metaphase chromosomes have divided but probably because of the lack of a spindle they have not separated. About $\times 1600$.

11 Section of a normal dividing cell from mammary gland of the mouse, centrifuged for 1 hour; although the chromosomes have been forced to the centrifugal side of the cell they have remained apart and have each divided; a large vacuole occupies the opposite side. About $\times 1600$.

12 Section of a rat sarcoma cell, centrifuged for 1 hour; although the chromosomes have divided no displacements have occurred. About $\times 1600$.

13 Section of a cell from a mouse adenocarcinoma, centrifuged for 1 hour; the chromosomes have divided, some even resembling the tetrads seen in divisions of the germ cells. No displacements are visible (compare with normal cell shown in fig. 11). About $\times 1600$.

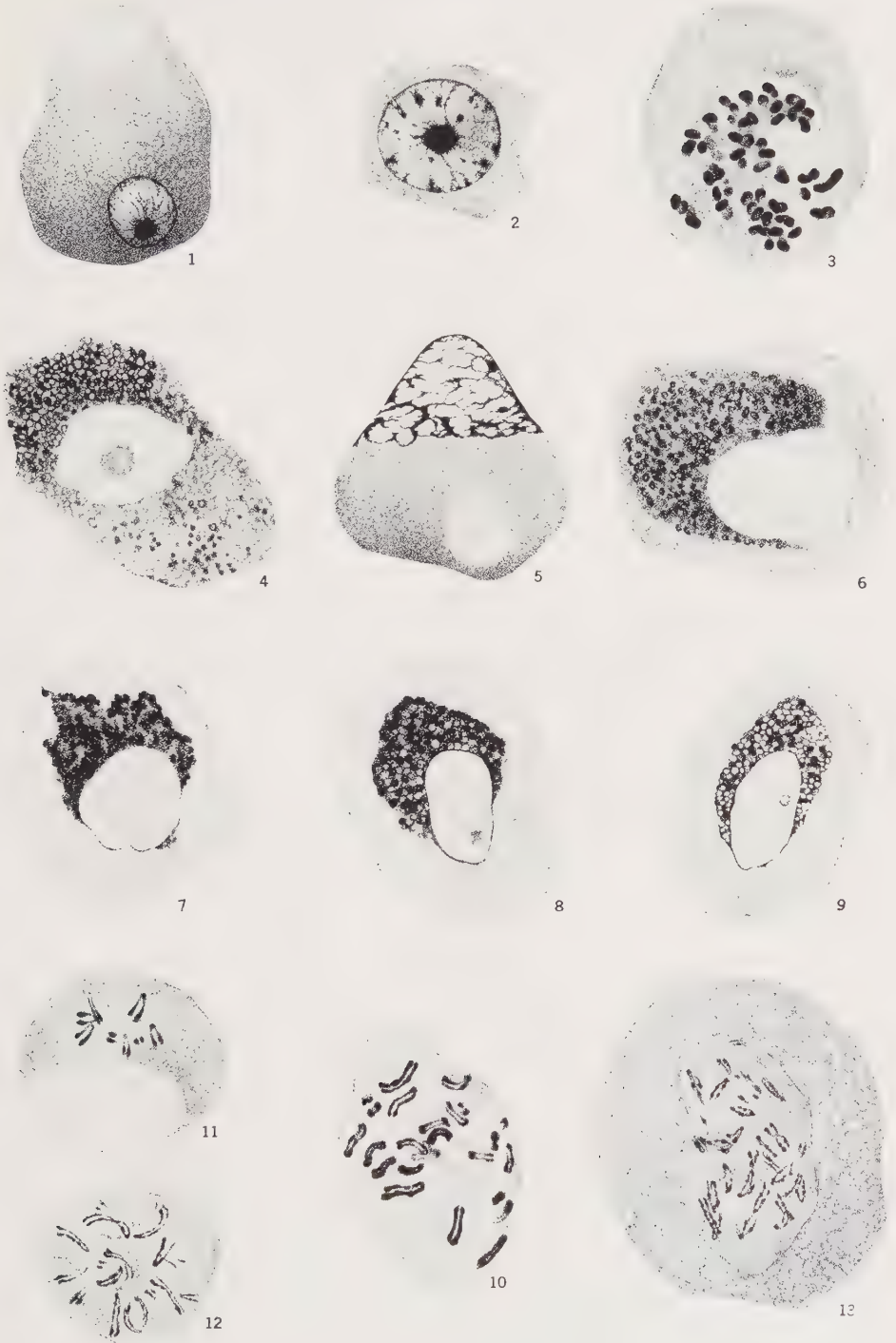


PLATE 2

EXPLANATION OF FIGURES

14 Section of adrenal cortex, centrifuged 1 hour, showing displacement of cellular contents. $\times 1000$.

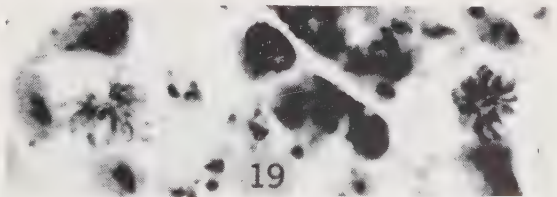
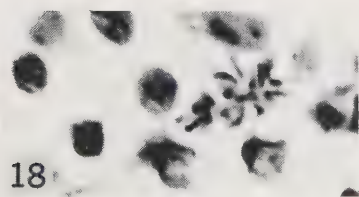
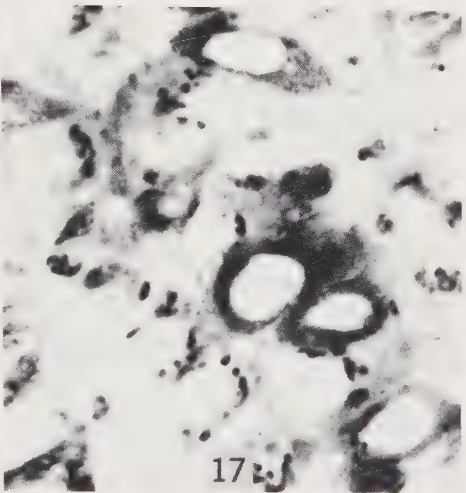
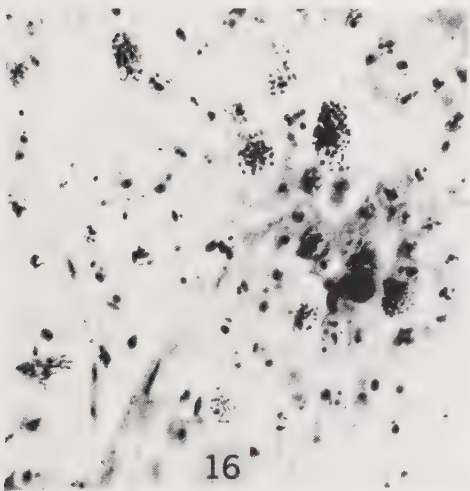
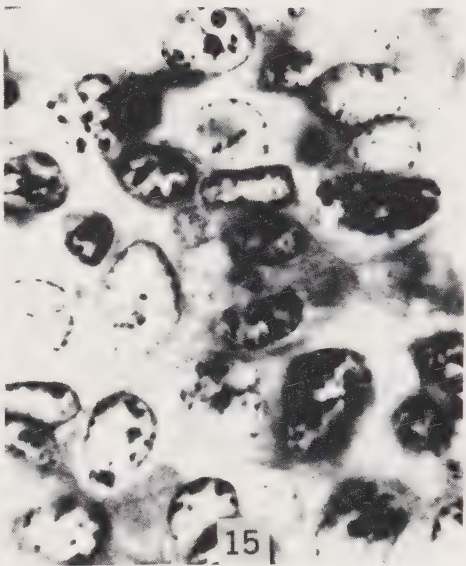
15 Section of rat carcinoma implanted in adrenal gland (that referred to in fig. 14) and centrifuged together with it for 1 hour, showing no perceptible displacements. $\times 1000$.

16 Section of rat carcinoma centrifuged for 1 hour showing an abundance of undisplaced metaphase chromosomes (indicated by the groups of black dots) in one microscopic field. $\times 430$.

17 Golgi material (in black) in a section of mouse adenocarcinoma centrifuged for 1 hour; the Golgi has the same appearance as in uncentrifuged material. $\times 1000$.

18 Section of mouse adenocarcinoma centrifuged for 1 hour, showing that the chromosomes still remain well apart. $\times 1000$.

19 Section of rat sarcoma, centrifuged for 1 hour, showing two sets of metaphase chromosomes. In each group certain of the chromosomes show faintly the double condition referred to in the text. $\times 1000$.



INTRANUCLEAR CRYSTALS IN THE HEPATIC CELLS OF CANIDAE—WOLVES, FOXES, JACKALS AND NON-DOMESTIC DOGS

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ONE PLATE (TWELVE FIGURES)

In a previous study (Weatherford, '38) on intranuclear crystals in the hepatic cells of the dog, variations were noted in the number of nuclei with crystals—0 to 2.13%—an average of 1.03%. Except in the proximal convoluted tubules of the kidney—a finding shared by Marchand (1887 and '09), Grandis (1889), Brandts ('09), Berg ('29), and Nicolau and Kopciowska ('36)—intranuclear crystals were not observed in other tissues of the dog. Grandis (1889) was unable to find intranuclear crystals in the frog, turtle, pigeon, young rat, rabbit (normal and dead from inanition), sheep, cow, young cat, pig and man.

The only mention of intranuclear crystals in hepatic cells other than in the dog, so far as I am aware, was by Berg ('34) in a fox (species not given), an observation suggesting that these crystals may be found in still other Canidae.

I am greatly indebted to Dr. Herbert Fox, pathologist to the Zoological Society of Philadelphia, who sent me the blocks of liver of representative Canidae, which made this study possible. The Canidae examined were: prairie wolf (*Canis latrans*) and gray fox (*Ucyon cinereo-argenteus*) two specimens, American red fox (*Vulpes fulva*), Fennec fox (*Fennecus zerda*), North African jackal (*Thos anthus*), Cape hunting dog (*Lycaon pictus*), and the bush dog (*Icticyon venaticus*)

one specimen each.¹ The material, already embedded in paraffin, was sectioned 5 μ thick and mounted serially. Sections on alternate slides were stained with hematoxylin and eosin, and with Heidenhain's 'azan.'

OBSERVATIONS

Forty-five thousand nuclei are counted—5000 in each liver, 1000 in a section—from each of the Canidae examined. The findings are presented in table 1.

As in domestic dogs, the number of nuclei containing crystals is variable. Even members of the same species

TABLE 1
A tabulation of the number of nuclei containing crystals in the livers of representative Canidae

	SPECIMEN	NUCLEI COUNTED	NUCLEI WITH CRYSTALS	PER CENT CRYSTALS
1	Prairie wolf	5000	7	0.14
2	Prairie wolf	5000	15	0.30
3	Gray fox	5000	26	0.52
4	Gray fox	5000	13	0.26
5	American red fox	5000	4	0.08
6	Fennec fox	5000	49	0.98
7	North African jackal	5000	10	0.20
8	Cape hunting dog	5000	5	0.10
9	Bush dog	5000	10	0.20

show considerable differences in crystal content. It is noted that in the two specimens of prairie wolf one contains more than twice the number of nuclei with crystals than the other: the percentages, however, are low in both wolves. The same is true of the two specimens of gray fox, although in each of these the percentages of nuclei enclosing crystals are higher than in the prairie wolves. In the livers of two non-domestic dogs—the Cape hunting dog and the bush dog—the crystal content is extremely low; in fact the percentages of nuclei with crystals are considerably lower than in the average domestic dog. There are even fewer than reported in a previous study on four pure bred Dalmatian dogs—

¹ The terminology follows suggestions from Dr. Glover M. Allen, as perhaps that most generally accepted today.

the breed consistently showing low counts of nuclei with crystals. The single specimen of Fennec fox shows the highest count—0.98%—most nearly approaching the average found in the domestic dog.

Crystallographically the intranuclear crystals are similar in all Canidae. They are prismatic, with hexagonal cross sections, and occupy clear areas or vacuoles within nuclei. Despite similarities in crystal form, species differences are observed. In some species the crystals are short and broad, while in others they are long and narrow. The longest crystals found, however, are usually shorter than many of those seen in livers of domestic dogs. Seldom do they have lengths equal to the diameters of the nuclei enclosing them. In the foxes the crystals are narrow and often occur multiple in the same or in separate clear areas, while in the jackal they are wider, generally single, sometimes elongating the nucleus. Only small crystals are found in the Cape hunting dog. The largest single crystal observed in any of these Canidae is in a specimen of American red fox where it distends the envelope surrounding the vacuole enclosing it into an elongated ellipse nearly equal to the diameter of the cell (fig. 4).

Crystals are observed in nuclei of several sorts—those of average size and good staining, large and pale ones, and small and dark staining ones. Differences in physiologic and mild pathologic conditions do not appear to affect the crystals. They are seen in cells with cloudy swelling, pigmentation, and in areas of focal necrosis. No crystals are found, however, in regions showing more extensive pathology.

Besides the intranuclear crystals some nuclei display homogeneous, rounded, ovoid and irregular bodies. These bodies, while usually occupying separate clear areas, may be found in the same areas with crystals. The homogeneous bodies are present with greater frequency in the nuclei of hepatic cells in some Canidae than in others. They occur abundantly in the prairie wolf and Cape hunting dog but are not quite so numerous in the red fox and North African jackal. In the Cape hunting dog, the homogeneous bodies are observed

as often as the intranuclear crystals, and in some counts of 1000 nuclei more often. Significantly, homogeneous bodies are met with but rarely in both nuclei of binucleate cells, and a similar condition prevails for the intranuclear crystals. Some nuclei exhibit an apparently empty clear area or vacuole. Whether this is a real condition or one resulting from cutting is not determined.

The sizes of the homogeneous intranuclear bodies vary; some are small, no larger than a true nucleolus, while others are observed having one-third to one-half the diameter of the nucleus. Like the crystals the homogeneous bodies do not fill completely the clear areas enclosing them.

Intranuclear crystals and homogeneous bodies stain similarly. Both stain with acidic (eosin) and basic (azo-carmin) dyes. As in livers of domestic dogs, which were stained with a variety of dyes, there is observed neither pronounced acidophilia nor basophilia.

The development of intranuclear crystals is studied best in the foxes and the Cape hunting dog. In the livers of these Canidae marked variations in crystal size are displayed, ranging from dispersed granulations near the limits of distinct microscopic resolution to well-formed crystals as long or longer than the diameter of a nucleus. Regardless of size the hexagonal prismatic shape prevails in all crystals.

DISCUSSION

Observations show the presence of intranuclear crystals and homogeneous bodies in the hepatic cells of nine members, representing seven different species of the family Canidae.

Berg ('34) discussed possible relationships between homogeneous intranuclear bodies and crystal formation. He assumed that material for the crystals wandered in through the nuclear membrane, concentrated in vacuoles as fluid droplets, and formed homogeneous bodies from which the crystals arose. Vacuoles were stated to be present in hepatic cell nuclei of forms other than the dog, both cold blooded animals and mammals, and it was conjectured that a lack of concentration or the presence of some disturbing substances hindered

crystallization in these cases, though the general metabolic conditions might be similar. Deficient concentration in the fluid droplets of the hepatic cell nucleus may account for the condition, very noticeable in the Cape hunting dog, where the homogeneous bodies are especially numerous and the intranuclear crystals few. It is impossible, however, to state whether these homogeneous bodies have any relation to the intranuclear inclusion bodies, presumably produced by filtrable viruses, described by Cowdry and Scott ('30) and Nicolau and Kopciowska ('36) in the hepatic cells of dogs, though the latter authors on not too secure a basis, similarity in staining, sought to derive the intranuclear crystals from the inclusion bodies.

From studies of illustrations, in a number of papers, depicting intranuclear inclusion bodies, I believe that while in some cases the figures show similarities to the homogeneous bodies seen in hepatic cell nuclei of Canidae, in others they present differences. Ludford ('30) stated: "The intranuclear virus bodies . . . are remarkably alike. Although some occasionally stain faintly basophil, the majority stain readily with acid dyes." Intranuclear crystals and homogeneous bodies stain with either acidic or basic dyes. Even with hematoxylin and eosin, when sections are stained deeply with hematoxylin the crystals take the basic dye and when stained less deeply the acidic dye. All of which leads me to think that the staining of both of these formations is possibly one of physical adsorption.

Cowdry ('28) has given some details of microchemical studies on intranuclear inclusions and while showing results, in part, comparable to those of Berg ('29) and mine ('38) on intranuclear crystals, they do not prove conclusively that these two are of the same material. More work should be done on both to extricate the problem, and the suggestions of Ludford ('30) might be followed profitably. The use of the ultracentrifuge, as by Lucas ('36) investigating intranuclear inclusion bodies, should be tried also in the study of intranuclear crystals.

Some of the earlier investigators, largely because of similarities in staining, considered the intranuclear crystals as

hemoglobin or a derivative. Crystallographically the intranuclear crystals in livers of domestic dogs and in other members of the family Canidae are dissimilar to hemoglobin.

Reichert and Brown ('09) studied the crystallography of hemoglobin in twelve members of the family, ten distinct species and two varieties or crosses. A general type of crystal was observed common to all species. It was described as more or less an elongated prism with a diamond-shaped cross section and usually strongly striated longitudinally. While the ratio of length to thickness of the crystals varied, both in the same and in different species, the crystallographic characters were found to be similar.

Because of the differences in form of hemoglobin and intranuclear crystals in all the observed Canidae, and the absence of iron in the ash of the intranuclear crystals in dogs—pointed out by Grandis (1889), Berg ('29), and Weatherford ('38)—it is unlikely that iron will be found in similar intranuclear crystals in other members of the family. Therefore it is concluded that the intranuclear crystals are not hemoglobin.

A. Hunter and his co-workers ('14 and '19), in their comparative studies of purine metabolism in various representative mammals, reported 'uricolytic indexes' of 98, 97, 96 and 32, respectively, for the domestic dog, prairie wolf (coyote), dingo, and Dalmatian dog. This index is the ratio of the output of allantoin nitrogen to the sum of the output of allantoin and uric acid nitrogen.

From counts of hepatic cell nuclei in the present study, and in an earlier one on dogs, it is seen that those species with the highest percentage of nuclei containing crystals have the highest 'uricolytic index,' and those with the fewest crystals the lowest index. These findings suggest a probable relationship between purine metabolism and crystal formation.

Perhaps long domestication of dogs, with attendant metabolic changes, accounts in part for the greater power of crystallization and therefore the more numerous and usually larger intranuclear crystals than are observed in other Canidae.

SUMMARY

1. Intranuclear crystals are observed in the hepatic cells of nine individuals, representing seven species of Canidae. The number of nuclei with crystals varies in the different species from 0.08% in the American red fox to 0.98% in the Fennec fox.

2. In all members of the family the crystals are presented as hexagonal prisms within clear areas or vacuoles. Crystal sizes range from the minute to those long enough to distort the nucleus.

3. In some nuclei, homogeneous, rounded, ovoid and irregular bodies are found, inclosed in separate or the same vacuole with crystals. These bodies are observed more often in some Canidae than in others.

4. A comparison of the crystals with what is known of the crystallography of hemoglobin in Canidae indicates that the two are not the same.

5. The observations on the intranuclear crystals, suggestions from the 'uricolytic index,' and previous experimental studies lead to the conclusion that there is a relation between purine metabolism and crystal formation.

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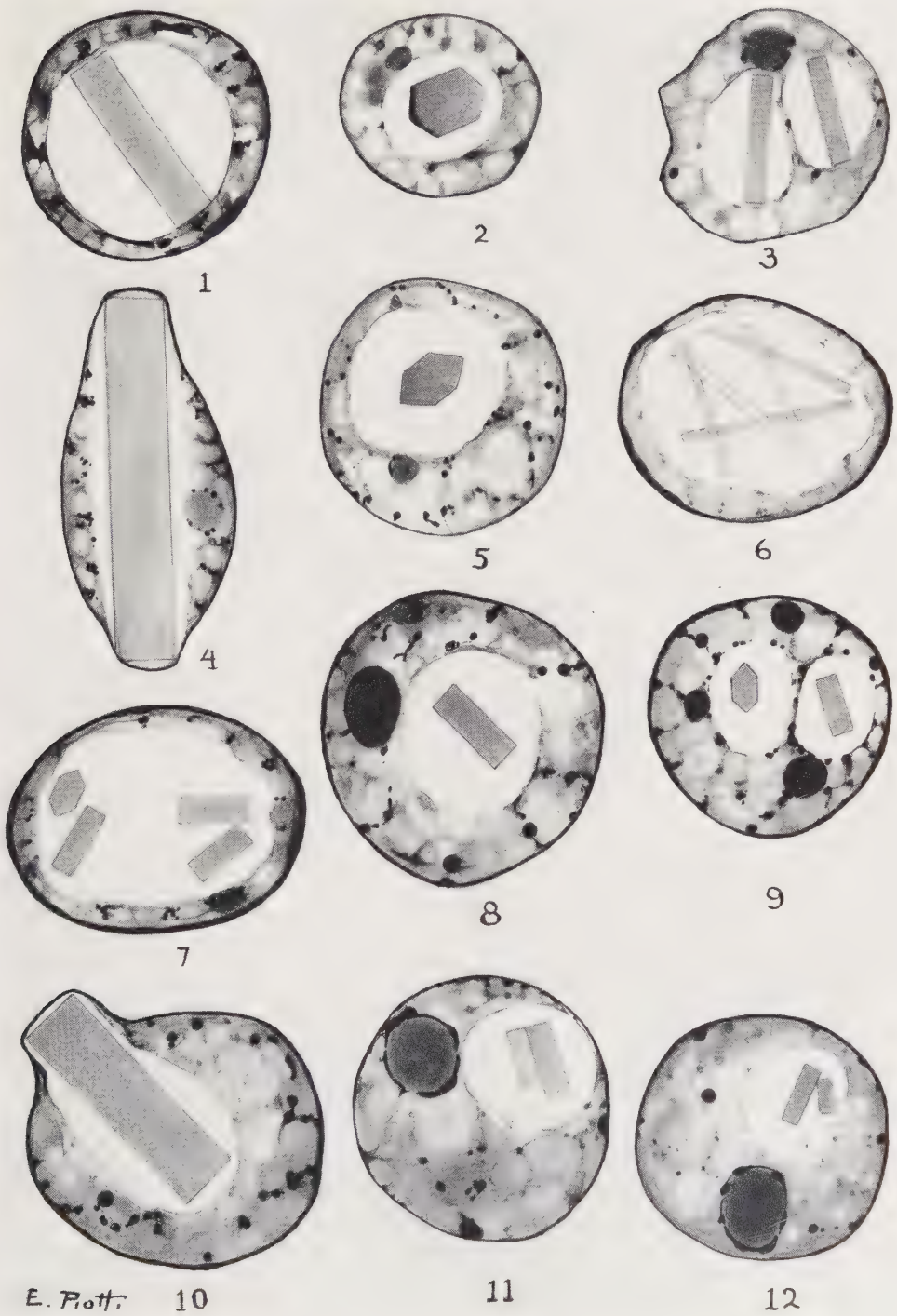
PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 12. Drawings of nuclei and contained crystals showing prismatic faces and hexagonal cross sections. Observe the clear area surrounding the crystals.

- 1 to 3 Prairie wolf (*Canis latrans*).
- 4 to 5 American red fox (*Vulpes fulva*).
- 6 to 7 Fennec fox (*Fennecus zerda*).
- 8 to 9 Gray fox (*Urocyon cinereo-argenteus*).
- 10 North African jackal (*Thos anthus*).
- 11 Bush dog (*Icticyon venaticus*).
- 12 Cape hunting dog (*Lycan pictus*).

All figures are drawn in outline with a camera lucida, details free-hand. The lens combination is a Zeiss apochromatic oil im. obj., 1.5 mm., N.A. 1.3 and $\times 30$ comp. oc., $\times 3700$.



STUDIES ON BONE MECHANICS IN VITRO

II. THE ROLE OF TENSION AND PRESSURE IN CHONDROGENESIS

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TWO TEXT FIGURES AND TWO PLATES (TEN FIGURES)

INTRODUCTION

There is general agreement that the structure of lamellar bone and especially the arrangement of spongiosa trabeculae, is adapted to the function of the bone and undergoes changes in response to altered mechanical conditions. The mechanical functions of finer bone units, such as osteons and lamellae, however, are not clearly understood.

In its histogenesis lamellar bone usually replaces coarse-fibered bone. Why this replacement occurs and how, if at all, it is correlated with mechanical stresses, is practically unknown. In ectopic ossification a primary formation of lamellar, as well as of coarse-fibered bone may occur. In and around tubercular lesions of human beings, for instance lamellar bone is often primarily formed. On the other hand, in the inflamed mammary gland of dogs coarse fibered bone usually appears first and is later replaced by lamellar bone. In neither case do obvious exogenous, mechanical forces influence the ossification and the changes in bone structure and the different modes of histogenesis appear to be unrelated to functional stresses. Other ectopic ossifications such as riding bones or the ossified tendons of birds and kangaroos are, however, obviously related to mechanical stresses. Thus, although not essential factors in ossification, mechanical forces may play a part in the process. With regard to the development of cartilage in callus formation and its replacement by bone some evidence is available that these processes too may be influenced by mechanical factors.

The literature abounds in contradictory views about the action of mechanical forces in skeletogenesis. Some authors believe that either continuous or intermittent pressure promotes ossification, while others hold that pressure causes resorption of bone. Divergent views are also expressed about the action and effect of tension on ossification. Some authors assume that tension acts directly on the osteogenic tissue, while others believe that it acts indirectly by affecting the blood vessels, the nutrition of the tissue or the release of calcium.

There is thus no definite information about either the importance or mode of action of mechanical factors in skeletogenesis. This is partly due to the fact that in the whole organism it is almost impossible to determine the exact stage of differentiation of the experimental tissue at different stages of an experiment. Experiments *in vitro* (Glücksmann, '38) have shown that this may be an extremely important factor since it was found that the effect of pressure on ossifying tissue depends entirely on the stage of differentiation and in particular on the degree of calcification of the developing bone, when the pressure is first applied.

In studies of the developmental mechanics of bone, tissue culture offers many advantages as compared with experiments *in vivo*. The development and behavior of the experimental tissue can be observed in the living state and the best stage for fixation for histological examination can be easily ascertained, while such general factors as vascularization, humoral and nervous influences are excluded. Moreover the tissue is much easier to manipulate and the mechanical conditions are much more readily controlled *in vitro* than *in vivo* where they are complicated by the pressure and action of muscles. In the experiments described in the present communication, cultures of osteogenic tissue have been used as living models with which to test the effects of pressure and tension on chondrogenesis. Later communications are intended to deal with the effect of mechanical factors on existing cartilage and with their role in bone formation.

MATERIAL AND METHODS

Tissue culture technique. Periosteum, perichondrium and cartilage from the metatarsals and phalanges of 7- to 13-day chick embryos were used as experimental material since previous work (Fell, Roulet and others) had shown that such tissue ossifies in vitro. In some experiments, as will be described later, the explants were combined with rib fragments from 17-day chick embryos. The cultures were grown by the usual hanging-drop or watch glass method for periods ranging from 2 to 12 days in a medium composed of 1 drop of fowl plasma mixed with 2 drops of extract of a 10-day chick embryo. The tissue was transferred to fresh medium every 2 or 3 days. Each explant was either drawn or photographed at the beginning and end of the culture period.

Histological methods. The cultures were fixed in Zenker's, Susa or Carnoy's solution and when ossified were decalcified in formol-nitric acid. Serial sections were cut and stained with azan, haematoxylin and eosin or by Hansen's or Wilder's method.

Methods of applying mechanical forces. Pressure and tension was applied to the tissue in one of three ways: 1) direct application of pressure by implanting the tissue between two ribs in culture; 2) indirect application of pressure by placing barriers in the direction of expansion of the growing explant; 3) direct application of tension by using the ordinary surface tension of the culture medium.

1. *Direct application of pressure by implantation between ribs.* As described in a previous paper (Glücksman, '38), explants of two rib fragments when connected by their intercostal muscles approach each other in culture, so that tissues implanted between them are subjected to pressure. In the present study twenty-six experiments of this nature were made.

2. *Indirect application of pressure by barriers placed in the direction of expansion.* Long bone rudiments grow readily in culture and elongate considerably. This expansion can be retarded or prevented if sufficiently heavy barriers are placed

at either end of the rudiment. The long bone thus grows against a resistance i.e., under pressure.

Other metatarsals or phalanges were used as barriers. In figure A, *a* and *b*, two such combinations of phalanges are seen. The middle rudiment in both is prevented from elongating further as soon as the cartilage comes into contact with the barriers. If, however, combinations with barriers in position *a'* or *b'*, figure A, are used the expansion of the middle rudiment is inhibited on one side only and the rudiment is thus forced to bend. This effect is illustrated by figure 1, in which the left half of the upper epiphysis of

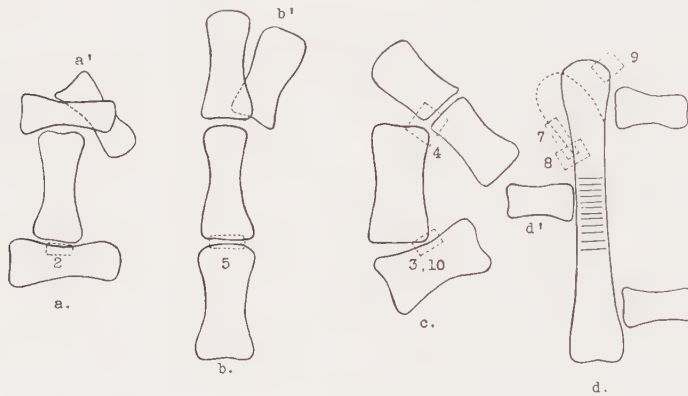


Fig. A shows types of combinations of bone rudiments used in experiments in which pressure was applied to periosteal and perichondrial tissue. The outlines indicate the areas illustrated in figures 2 to 5 and 7 to 10.

the middle phalanx is much larger than the right half and the right side of the diaphysis less concave than the left side; the surface of the lower epiphysis is flattened where it is in contact with the barrier. The same type of effect is produced by the combination figured as *c* in figure A. In combinations like that shown in *d* a slightly ossified metatarsal was used and smaller phalanges were placed at right angles to its long axis on one or both sides of each epiphysis. Such metatarsals bend easily at the border between the ossified and unossified part of the diaphysis, as indicated by the dotted line in the diagram.

Pressure is exerted not only on the middle rudiment, but also on the barriers themselves. Thus in combinations like that shown in *c*, figure A, the expanding middle rudiment exerts pressure on all the other cartilages. This pressure acts at first on the perichondrial and periosteal membrane of the barriers. The longitudinal fibers which largely compose this membrane are inserted in the epiphyses at either end so that their mobility is restricted. Under pressure, the more mobile, peripheral fibers are pushed aside while the inner fibers are trapped and compressed. The less viscous inter-fibrous material is forced away from the site of pressure raising a bulge in the surrounding tissue. In the individual collagen fibers a similar displacement of material occurs as each fiber consists of fibrils embedded in a ground substance. Thus two areas may be recognized, viz., the site of pressure and the region to which material has been displaced; the latter is referred to in this communication as the site of displacement.

When the epiphysis of a metatarsal is bent (fig. A, *d*) it causes the perichondrial fibers to become detached from the cartilage on the concave side of the metatarsal.

Fifty-four experiments with 'barriers' were made, eighteen of which were of type *a* or *a'*, figure A, ten of type *b* or *b'*, fifteen of type *c* and eleven of type *d* or *d'*.

3. *The mechanical influence of the surface tension of the culture medium.* The epiphyses of metatarsals become flattened in hanging-drop cultures if they are near the periphery of the culture medium. This is due to the surface tension stresses in the medium. While the center of the clot liquefies, the periphery loses fluid and shrinks thus increasing the surface tension stresses toward the margin.

A cross section of an epiphysis flattened in this way is figured in figure B, *a* and *b*. Dots around the epiphysis represent cross sections of the perichondrial fibers. The surface tension stresses act at right angles to the long axis of the fibers and tend to pull them away from the cartilage. The flattening of the epiphysis is caused mainly by restriction of cartilage-formation to the direction of the tension stresses.

Whether there is any reduction in the original diameter of the epiphysis is uncertain, but if so it plays only a minor part in the flattening process.

Ten experiments of this type were made. In addition, many of the fifty-four experiments described in section 2, incidentally showed the same effect of the medium on the epiphyses.

RESULTS

Cartilage regularly developed in the periosteum or perichondrium both at the site of pressure and at the site of displacement, whether the displacement was produced by pressure or by tension. The histology and histogenesis of this

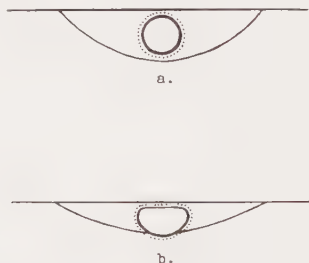


Fig. B shows diagrammatically a cross section through the epiphysis of a bone rudiment in the periphery of the culture medium of a hanging-drop culture: (a) at the beginning of the cultivation period and (b) 2 or 3 days later.

cartilage will be described first after which an attempt will be made to analyze the mechanism of its formation.

1. *Cartilage formation at the site of pressure.* Cartilage appeared at the site of pressure in both perichondrium and periosteum and might be of either the small- or large-celled type. (The small-celled cartilage is characterized by the size of its cells which are surrounded by a very pronounced, often still fibrillar capsule, and by the small amount of intercapsular ground substance. In the large-celled cartilage the cells are surrounded by a thin capsule and separated from each other by well-developed intercapsular ground substance. The latter type is not identical with hypertrophic cartilage.)

Thus in one combination of type *a* figure A, a bowl of small-celled cartilage developed in the diaphysial periosteum of the lower 'barrier' rudiment (fig. 1), fitting the adjacent epiphysial cartilage of the middle rudiment like an acetabulum. It was interesting that in this specimen the cartilage had been formed in the periosteum outside the bone which had already been deposited as a thin layer between the periosteum and the cartilaginous diaphysis. In another combination of type *a* a similar cup-shaped mass of cartilage had been formed by the periosteum external to the bone, but in this case the cartilage was of the large-celled type (fig. 2).

Small-celled and large-celled cartilage sometimes occurred together. For example in a combination of type *c*, figure A, the usual bowl-shaped nodule of cartilage had been formed in the periosteum of the 'barrier' (fig. 3). At the site of pressure the cartilage was of the small-celled type but at the site of displacement, where the distance between the two bone rudiments was wider, the cartilage cells were larger.

Perichondrium subjected to pressure behaved in a similar way. To test the effect of pressure on the epiphyseal perichondrium, two phalanges were left with the joint between them intact and this joint region was then used as a barrier in a combination of type *c*, figure A. Small-celled cartilage formed in the perichondrium at the site of pressure (fig. 4) thus causing the three phalanges to fuse at the area of contact. The new cartilage was distinguishable from the pre-existing cartilage by its smaller cells. Cartilage also formed across the joint between the two adjacent phalanges of the 'barrier,' and fused with the new cartilage at the site of pressure. In this way a triangular field of new cartilage was formed between the three phalanges (fig. 4).

Cartilaginous fusion at the site of pressure in the perichondrium sometimes tended to obscure the formation of new cartilage. The first three examples described above are so clear because the new cartilage developed in the periosteum and was separated from the pre-existing cartilage by a thin layer of bone. If, however, new cartilage is formed by a thin

layer of perichondrium separating two phalanges as in the case of *c* in figure 1, the new cartilage is only just distinguishable from the old. Other specimens showed all stages between this condition and a cartilaginous fusion like that shown in figure 4. Complete or partial union was particularly common in combinations of the type *b* and *b'* in figure A. A typical stage in the complete union of two epiphyses by the formation of new cartilage is shown in figure 5. Before being combined the two phalanges had been carefully separated from each other. After they had again been placed in apposition the perichondrium of either epiphysis formed new cartilage at the site of pressure which varied in character from a very compressed, small-celled type in the middle of the joint region to a fairly large-celled type toward the periphery of the joint. The outlines of the pre-existing cartilage were still just visible.

2. *Cartilage formation at the site of displacement.* When cartilage developed in a site of displacement immediately surrounding a site of pressure, it did so in direct continuity with the cartilage which appeared at the site of pressure (fig. 3). As already stated, the plate of new cartilage was usually thicker at the site of displacement than at the site of pressure, and the cartilage cells were correspondingly small and compressed in the thin region and larger and more rounded in the thicker part.

Cartilage formation at the site of displacement caused by pressure was clearly seen in experiments in which the pressure was applied by two approaching ribs. An example of this type of result is shown in figure 6, where half a phalanx had been implanted between the ribs at an angle of about 45°. During cultivation the ribs had approached each other in the usual way, and the phalanx had gradually been shifted to a position nearly parallel to the ribs, so that the cut end had moved from its original point of contact with the right rib toward the left rib. During this movement the perichondrial tissue on the left of the phalanx became squeezed out to the position *a* where it formed cartilage.

Displacement of perichondrium by tension stresses had the same effect as displacement by pressure. In combinations like d or d' in figure A, the metatarsal was bent, and the perichondrium detached itself from the shaft. The space between the shaft and the perichondrium became filled with new cartilage, the development of which proceeded from the epiphysis toward the diaphysis and from the perichondrium toward the pre-existing cartilage (fig. 7).

Displacement of the perichondrium by bending or flattening of the epiphysis sometimes disorganized the perichondrium by releasing its normal tension. Under such circumstances chondrogenesis spread into the surrounding tissue (figs. 8 and 9).

3. *Mechanism of cartilage formation.* In the experiments described above, cartilage of the large-celled type developed both at the site of pressure and at the site of displacement, but the mechanism of its formation was not the same in the two cases. Thus at the site of pressure, small-celled cartilage preceded the appearance of the large-celled type (fig. 5). At the site of displacement, on the other hand, small-celled cartilage never developed even in the early stages of chondrogenesis (figs. 7, 8 and 9), and the large-celled type only was formed.

The first effect of pressure on perichondrial and periosteal tissue was to flatten and compress the cells and fibers. The fibers then condensed and came into contact with each other except where they were separated by the narrow, elongated cells (a in fig. 10). The fibers surrounding the cells fused, thus forming pericellular capsules, and elsewhere broke down (figs. 5 and 10). After the capsules had been formed, the cells tended to shorten and become rounded. At the same time a number of degenerate cells and broken down collagen material were seen. The dense capsules were not permanent but gradually disintegrated and became less conspicuous, although their inner outlines were preserved. The amount of intercellular ground substance increased; remnants of fibers could still be demonstrated in the ground substance by silver

impregnation. There was therefore direct developmental continuity between the cartilage capsules of the early stage (figs. 5 and 10) and those of the later stages (figs. 2 and 3). Enlargement of the cells continued simultaneously with the increase in the matrix. All stages in the process of chondrogenesis were often present in the same explant (fig. 5).

The process of cartilage formation at the site of pressure was thus characterized by the primary formation of capsules and the secondary formation of intercapsular ground substance.

As described above, at the site of displacement the tissue usually bulged out at right angles to the original orientation of the fibers. The fibers or fiber bundles were not condensed but split up into very fine fibrils which soon broke down into short fragments. In this way an irregular network of short, fine fibrils originated containing the disintegrated remains of collagen material. This network then became impregnated with a cementing substance which filled the meshes and the reticulum broke down still further. Soon the whole ground substance appeared hyaline, the cells became rounded and the ground substance condensed around them to form capsules (fig. 9).

Cartilage formation at the site of displacement was therefore characterized by the primary formation of ground substance and the secondary formation of capsules.

Both the types of chondrogenesis described above are also seen in the normal embryo. Primary capsule formation occurs, for example, in the lateral parts of the epiphysis and the primary formation of ground substance in the early development of the diaphysis.

DISCUSSION

The results of the present experiments have shown that *in vitro* perichondrium and periosteum form cartilage under pressure. Benninghoff ('24), Moskoff ('33) and Krompecher ('37) observed a similar phenomenon *in vivo*. In experiments with dislocated ribs in dogs, Benninghoff found

that cartilage degenerated at the site of pressure but that in perichondrial tissue which was 'spezifisch deformiert,' cartilage developed at the site of displacement. Moskoff, in investigations on tracheal cartilage, confirmed these observations. Thus cartilage forms at the site of displacement in vivo as well as in vitro. In experiments on the influence of mechanical stresses on callus-formation in dogs, Krompecher found that the application of pressure caused cartilage to develop at the site of pressure, so that the influence of pressure on cartilage formation in vivo is essentially the same as in vitro.

Benninghoff interpreted the action of pressure on cartilage formation as follows. He assumes that fibrous systems are specifically deformed by pressure acting along the direction of the fibers. The fibrous network is split open so that the fibers form 'arcades' around the cells instead of running parallel to them and in their new position are directly transformed into cartilage capsules. A similar effect is assumed for tension stresses acting at right angles to the direction of the fibers.

This theory, which is accepted by Moskoff and Krompecher and which is applicable solely to cartilage formation at the site of displacement does not explain the results of the present writer's experiments in vitro. Although in the explants the fibrous network split up at the site of displacement capsules did not develop and the fibers merely disintegrated.

The formation of a cementing substance, essential for the development of hyaline matrix, is not an immediate result of the mechanical stress on pre-existing fibers but may be an indirect sequel to this effect of pressure. It is possible that broken down fibrous and cellular material may be utilized in the formation of ground substance; it is also conceivable that the presence of this material might in some way stimulate the cells to form matrix. On the other hand, the cells may be induced to secrete matrix as a direct result of being subjected to pressure.

That pressure is not an essential factor in cartilage formation has been shown by Fell in an experiment quoted by Murray; cartilage developed, obviously in the absence of pressure stresses in some parts of a teased limb-bud derived from a young chick embryo. The present writer's observation, described above, that cartilage spreads outward, in the absence of a restricting tension in the perichondrium, points to the same conclusion.

Fell, Roulet and others have already shown that periosteum is capable of forming cartilage as well as bone during cultivation in vitro. The experiments recorded in the present communication have demonstrated that mechanical factors may decide whether cartilage or bone shall develop in a tissue capable of forming both.

The direct effect of pressure on fibrous tissue is easily understood, but why this initial effect should be followed by the formation of hyaline ground substance remains obscure. Benninghoff's theory also makes no attempt to explain the problem of matrix formation. Pressure seems to initiate chondrogenesis mechanically, and in some yet unknown way to establish conditions which lead to the formation of ground substance.

I have pleasure in acknowledging my indebtedness to Dr. H. B. Fell for her helpful suggestions, critical advice and the interest she showed throughout this work; to the British Fund for German Jewry for a maintenance grant; to Mr. V. C. Norfield for the photomicrographs.



SUMMARY

1. Pressure causes cartilage formation in perichondrium and periosteum a) at the site of pressure and b) at the site of displacement.

2. Tension stresses acting at right angles to the direction of the fibers in perichondrium cause displacement of fibrous tissue and subsequent cartilage formation.

3. Two types of chondrogenesis can be distinguished: a) primary capsule formation and secondary appearance of ground substance and b) primary formation of ground substance and secondary appearance of capsules. The first mechanism is observed at the site of pressure, the second at the site of displacement.

4. In the absence of a restricting tension in the perichondrium, cartilage-formation spreads into the surrounding tissue.

5. Although pressure is not an essential factor in cartilage formation, when exerted on periosteum or perichondrium it initiates cartilage formation mechanically and sets up conditions favoring the formation of a hyaline ground substance.

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DESCRIPTION OF PLATES

All the figures are photomicrographs. Figures 2 to 5 and 8 to 10 are taken from areas outlined in figure A. Figure 7 corresponds to a mirror image of the area outlined in *d*, figure A. All the sections of cultures illustrated were cut parallel with the surface of the culture medium in the original culture vessel.

PLATE 1

EXPLANATION OF FIGURES

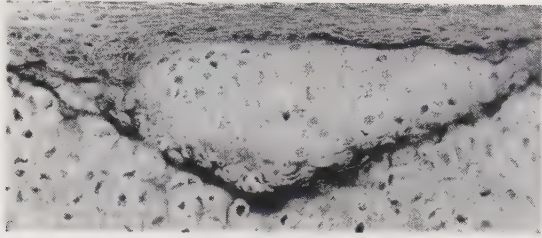
1 Section through a combination of phalanges derived from a 12-day chick embryo, cultivated by the watch glass method for 4 days. Compare *a'*, figure A. A bowl of small-celled cartilage (*a*) is formed in the periosteum of the upper 'barrier' rudiment. The left half of the upper epiphysis of the middle rudiment (*b*) is larger than the right half. New cartilage is seen at (*c*). Azan. $\times 30$.

2 Section through a combination of metatarsal and phalanges derived from a 12-day chick embryo, cultivated by the watch glass method for 8 days. Compare the outlined area in figure A, *a'*. Large-celled cartilage has appeared in the periosteum of the barrier rudiment at the site of pressure. Azan. $\times 170$.

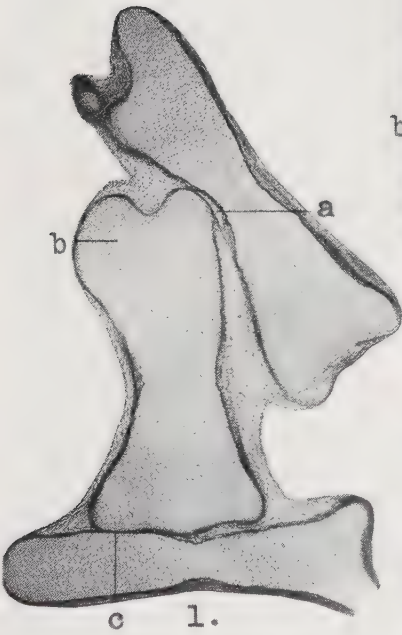
3 Section through a combination of phalanges derived from a 7-day chick embryo, cultivated by the watch glass method for 11 days. Compare the outlined area in figure A, *c*. Small-celled cartilage (*a*) at the site of pressure is continuous with large-celled cartilage (*b*) at the site of displacement. Wilder's method. $\times 210$.

4 Section through a combination of metatarsal and phalanges derived from a 7-day chick embryo, cultivated by the watch glass method for 11 days. Compare the outlined area in figure A, *c*. A triangular field of new cartilage joining the three phalanges is seen at (*a*). Azan. $\times 48$.

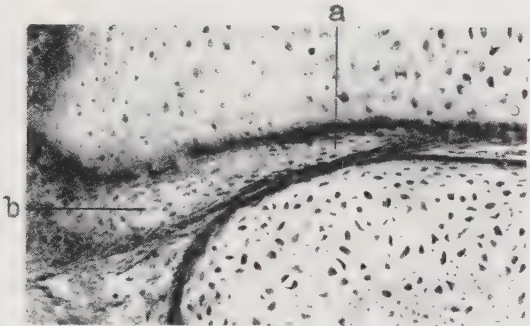
5 Section through a combination of metatarsal and phalanges derived from a 12-day chick embryo, cultivated by the watch glass method for 7 days. Compare the outlined area in figure A, *b*. In the middle of the joint area between two adjacent epiphyses small-celled cartilage (*a*) is seen merging into the large-celled type toward the periphery of the joint (*b*). Azan. $\times 240$.



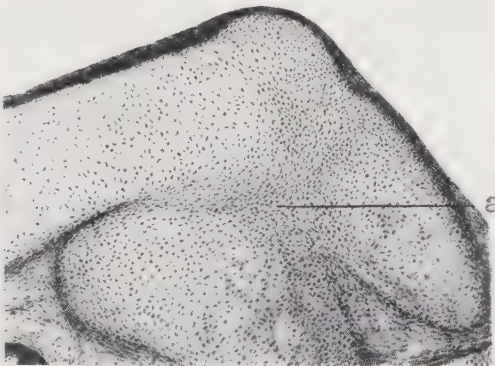
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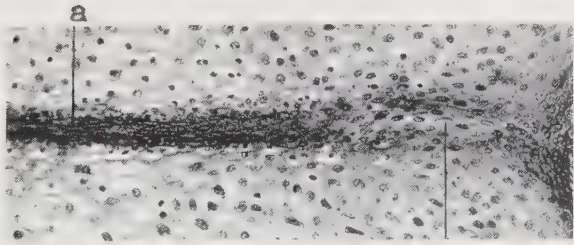
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PLATE 2

EXPLANATION OF FIGURES

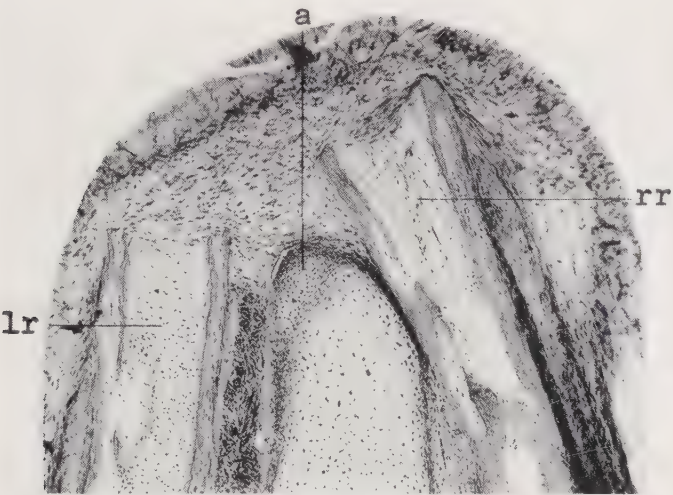
6 Section through a combination of ribs of a 17-day chick embryo and half a phalanx derived from a 13-day chick embryo, cultivated by the hanging-drop method for 7 days. Compare figure A, Glücksmann ('38). A nodule of new cartilage (*a*) has appeared at the site of displacement. *lr*, left rib; *rr*, right rib. Haematoxylin-eosin. $\times 40$.

7 Section through a metatarsal of an 11-day chick embryo, cultivated by the hanging-drop method for 8 days. Compare the outlined area in figure A, *d*. New cartilage is formed in the space between the cartilaginous shaft (*a*) and the detached perichondrium (*b*). Azan. $\times 280$.

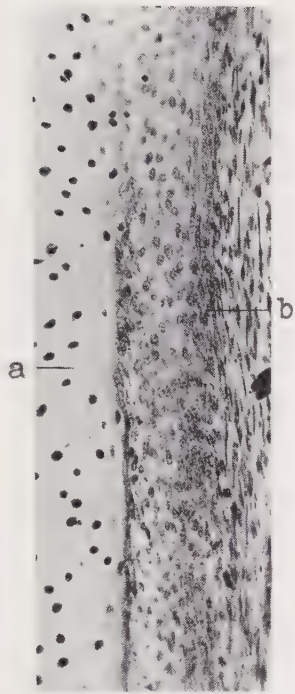
8 Section through a combination of metatarsal and phalanges derived from a 10-day chick embryo, cultivated by the hanging-drop method for 3 days. Compare the outlined area in figure A, *d*. The perichondrium has become detached and disorganized by the bending of the epiphysis. Cartilage formation spreads into the surrounding tissue. Azan. $\times 240$.

9 Section through a metatarsal of a 9-day chick embryo, cultivated by the hanging-drop method for 11 days. Compare figure B and figure A, *d*. Flattening of the epiphysis has caused a disorganization of the perichondrium and thus leads to a spreading of cartilage formation in the surrounding tissue. Azan. $\times 240$.

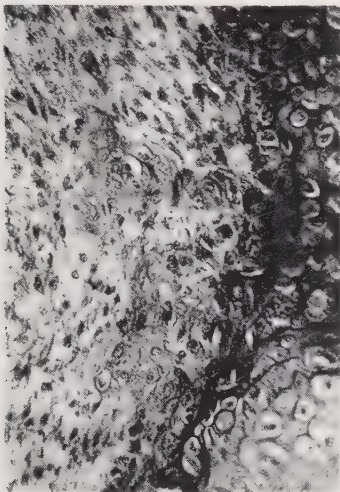
10 Section through a combination of metatarsal and phalanges derived from a 7-day chick embryo, cultivated by the watch-glass method for 12 days. Compare the outlined area in figure A, *c*. Compression of fibers and elongation of cells is seen at (*a*). Cartilage capsules appear at (*b*). Wilder's method. $\times 540$.



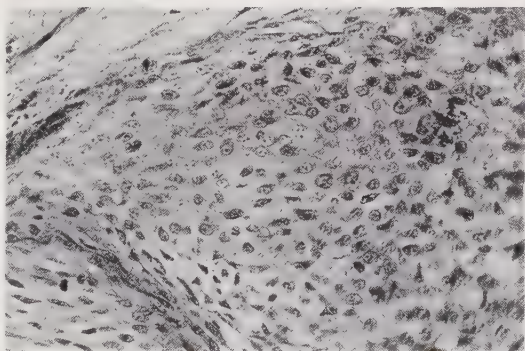
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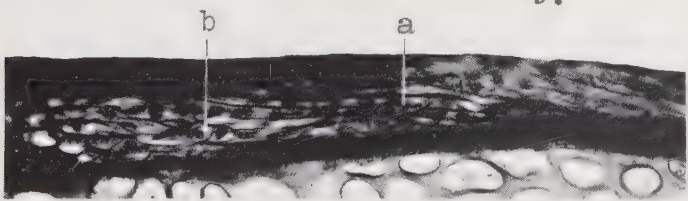
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THE MORPHOGENESIS OF THE HYPOPHYSIS CEREBRI OF THE DOMESTIC FOWL DURING THE SECOND AND THIRD WEEKS OF INCUBATION

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ELEVEN TEXT FIGURES AND TWO PLATES (FIVE FIGURES)

INTRODUCTION

Studies relating to the development of the hypophysis of the domestic fowl have been numerous and date back many years. However, in many of these studies, consideration of the hypophysis has been incidental to other interests of the observer, and no single investigator has given a complete account of the morphogenesis of the gland throughout the developmental period.

Interest in the structure of the hypophysis of birds has been revived recently by such papers as the abstract by De-Lawder, Tarr and Geiling ('34) who studied the chicken because of the "apparent absence of a well-defined pars intermedia either closely investing the pars posterior or invading the neural tissue in the form of strands of epithelial cells, as in the case of most mammals." Description of a similar condition in the porpoise has been provided by Wislocki ('29), and in the whale by Valsö ('34), and by Wislocki and Geiling ('36). Wislocki ('38) and Oldham ('38) have studied the hypophysis of the armadillo and find that in this form also there is absence of a discrete intermediate lobe.

Furthermore, it has been found (Wislocki, '38) that certain other Xenarthra (adult three-toed and two-toed sloths) do

not possess a pars tuberalis, while in the adult anteater a pars tuberalis is histologically demonstrable but with atypical topographical relations. These facts have heightened the interest of investigators in careful studies of the structure of this gland, which now appears to be more variable than had been supposed.

In an earlier study (Atwell and Sitler, '18) the morphogenesis of the hypophysis of the chick was traced by means of serial sections and numerous wax-plate reconstructions, to the end of the sixth day of incubation. At that time the interest of the authors was centered in discovering the first appearance of the pars tuberalis and in tracing its early development.

The writer is unaware of any recent investigation which has employed a series of plastic reconstructions to show the later stages of development of the hypophysis of the chick. It has seemed, therefore, that illustration and description of sections and models covering the second and third weeks of incubation and the first day after hatching would be of value as an aid to understanding the adult structure of this important gland.

MATERIAL AND METHODS

This study is based upon serial sections made in both sagittal and frontal planes from each of the following stages of development of the domestic fowl (*Gallus domesticus*): 7, 9, 11, 13, 15, 16 and 18 days of incubation and 1 day after hatching. Eggs of a homogeneous strain of White Leghorns were incubated in the laboratory. Day-old chicks of a similar breed were obtained from a commercial hatchery.

The entire head of the younger chicks, or the hypophysis region of older ones was fixed in aqueous Bouin's fluid or in 10% formalin. When necessary, decalcification was accomplished by 5% nitric acid or by Huber's fluid. After thorough washing the blocks of tissues were trimmed, dehydrated and embedded in paraffin. Serial sections were cut at 10 μ and stained with hematoxylin and eosin.

Wax-plate reconstructions of the hypophysis region have been made from the sagittal sections of five of these stages: i.e., 7-day, 11-day, 15-day, 18-day and 1 day after hatching. The model made from the 7-day chick was constructed at a magnification of 200 diameters; the remainder at 100 diameters.

Certain sections and four of the models have been chosen for representation in the accompanying illustrations.

OBSERVATIONS

In general shape the epithelial portion (*pars buccalis*) of the hypophysis of the chick makes definite changes during the period of development under consideration. At 7 days (figs. 1 and 12) the caudal portion of the gland lies parallel with the neural lobe and the brain floor while the rostral portion is arranged in the direction of the epithelial stalk. The two portions thus make an angle of approximately 90° with each other.

After the disappearance of the epithelial stalk the rostral portion of the gland arranges itself in the same general axis as the caudal portion. The result is that the epithelial part (exclusive of the *pars tuberalis*) assumes a shape very suggestive of a slipper (figs. 4, 5, 14, 15, 16). It can be seen readily from these figures that the rostral part is both thicker and wider than the caudal portion.

During all these stages and also in the 6-day embryo as pictured by Atwell and Sitler, the caudal and rostral parts are separated by a groove which is distinct on the ventral and lateral surfaces of the gland. This groove is occupied ventrally by a large anastomoses between the two internal carotid arteries and laterally by the arteries distal to the anastomosis (see especially figs. 1 to 7 and 12 to 14). In mid-sagittal sections the arterial anastomosis is seen to be in relation with the instep of the slipper (figs. 4 and 5).

The presence of the groove occupied by the internal carotid arteries results in a partial subdivision of the gland into two parts which it is proposed to call the rostral and caudal

divisions of the anterior lobe (figs. 12 to 16). Attention has already been directed to the fact that the rostral division is the larger.

After the disappearance of the epithelial stalk the long axis of the pars buccalis lies in a rostro-caudal direction. This



Figures 1 to 5. Diagrammatic drawings of mid-sagittal sections of the hypophysis region in chick embryos of the following stages of development: Figure 1, 7 days; figure 2, 9 days; figure 3, 11 days; figure 4, 13 days; figure 5, 16 days of incubation. The rostral end is at the right. Solid black, epithelial hypophysis and epithelial stalk; lined, brain wall and neural hypophysis; double outline, internal carotid artery. In figure 1 'X' indicates the part of the epithelial hypophysis which in many mammals becomes the pars intermedia. $\times 33$.

is in contrast with the human hypophysis in which for a considerable part of development the side-to-side dimension is the greatest (Atwell, '18).

The residual lumen. The cavity of the gland or the residual lumen of Rathke's pouch is still nearly complete at 7 days (fig. 1). It continues from the apex of the original pouch, near the neural lobe to the vicinity of attachment of the epithelial stalk. At 9 days (fig. 2) the cavity has been broken up by the growth of the gland into an isolated part at the extremity nearest the neural lobe and another, larger, part near the attachment of the epithelial stalk. By 11 days (fig. 3) the cavity has become so much subdivided that only small portions are to be seen. Thereafter only occasionally can a fragmentary remains be identified. In later stages it is entirely lacking. Isolated portions of the cavity which are to be found for a time in the tuberal processes are considered in the description of the pars tuberalis. See also Bruni ('15).

The method of disappearance of the residual lumen in the developing chick is thus seen to be different from that found in many vertebrates. In the rabbit and in man, for example (Atwell, '18, '26), the part of the lumen in the rostral part of the anterior lobe near the attachment of the epithelial stalk disappears first leaving a portion of the cavity at the apex which is more or less permanent, and which serves to separate the pars intermedia from the anterior lobe proper. In the rabbit, as in many other mammals, this part of the lumen persists throughout life. In man it is evident well into infancy or childhood and then is lost.

The pars intermedia. Two topographical criteria for identification of the pars intermedia during development have been: 1) Intimate relation with the neural lobe and, 2) separation from the anterior lobe proper by the residual lumen. To a certain degree both of these are met by the conditions found in the 7-day and certain younger chicks (fig. 1). Since, however, the union with the neural lobe is never very close and since the residual lumen disappears relatively early, no indication of a pars intermedia can be recognized after 7 or 8 days of incubation.

While the contact of the epithelial lobe with the neural lobe is never extensive or very intimate some regions of approximation may be seen in certain 9- and 11-day chicks. Two such regions are shown in figure 10. These rather close approximations seem to be transitory, however, for they are not seen in older stages where a moderately thick layer of connective tissue separates neural and buccal lobes (fig. 11).

The pars tuberalis. The earlier stages in the development of the pars tuberalis, including the first 6 days of incubation, have been traced by Atwell and Sitler ('18). At 7 days this portion is represented by two elongated buds with rounded ends (the tuberal processes, figs. 8 and 12). These are attached to the anterior lobe just in front of the groove which lodges the internal carotid arteries and their anastomosis. Thus they spring from the dorsal surface of the gland near the caudal limit of the rostral division. Attachment at this location is maintained throughout all the succeeding stages studied. At 7 days each tuberal process contains a small lumen (fig. 8) isolated from the rest of the hypophyseal cavity. By 9 days of incubation these small lumina have disappeared.

Scheme for figures 6 to 11. Solid black, epithelial hypophysis and epithelial stalk; lined, brain wall and neural lobe of hypophysis; stippled, cartilage of sphenoid; double outline, large arteries. All figures of this group are reproduced at a magnification of $\times 33$.

Fig. 6 Frontal section of the hypophysis region of a chick, 9 days of incubation, showing the relation of the anastomosis of the internal carotid arteries (int. c.) to the anterior lobe of the hypophysis.

Fig. 7 Paramedian sagittal section of the hypophysis region in a chick, 9 days of incubation. The rostral end is at the right. The relation of the internal carotid artery to the groove which separates the anterior lobe into rostral and caudal divisions is shown. The tuberal process (t. p.) is seen to arise from near the caudal end of the rostral division.

Fig. 8 Frontal section of the hypophysis region of a chick incubated 7 days, showing the tuberal processes (t. p.) in close proximity to the brain floor.

Fig. 9 Frontal section of the hypophysis region of a chick, 13 days of incubation, showing pars tuberalis (p. t.) flattened and rather closely applied to the brain.

Figures 10 and 11. Frontal sections of the hypophysis region of chicks incubated 11 and 18 days, respectively. Figure 10 shows two regions of approximation of neural and epithelial lobes (X). In figure 11 the neural and epithelial portions are separated by a considerable layer of connective tissue.

By 11 days of incubation (fig. 13) the two halves of the pars tuberalis are present as plates of tissue distinctly flattened against the brain wall. This condition is well illustrated by a frontal section from a 13-day chick (fig. 9). How these two halves continue to conform more closely to the brain floor is



Figures 6 to 11

shown in figures 14 to 16. They continue to spread out under the brain and their area in contact with the brain increases.

While the two halves of the pars tuberalis remain separate for a relatively long time when compared with the rabbit for example, they finally approach each other in the midline and

eventually unite. As in other forms this fusion takes place in two locations, one in front of the infundibular process and the other caudal to it. In the latter position each half sends a sharp caudal horn backward (fig. 16). These meet caudal to the infundibulum. The more extensive rostral portions of the pars tuberalis have approached each other in the mid-line as shown in figure 16 (18 days of incubation). Actual meeting and fusing of the halves is first seen in the day-old chicks of our series.

The epithelial stalk. The epithelial stalk may be seen in the 7-, 9-, and 11-day chicks (figs. 1 to 3). Its cavity is discontinuous at 7 days, and becomes subdivided into a greater number of parts during the next 4 days. In these stages a portion of the stalk containing a remnant of the cavity tends to be somewhat enlarged and cyst-like (figs. 2 and 3). After 11 days of incubation the stalk is no longer continuous although certain later stages show remnants of it. One 13-day chick shows an isolated mass of cells containing a cavity. This mass is located ventral to the developing sphenoid and near the pharyngeal epithelium. In one 15-day chick also may be seen groups of faintly-staining cells located in the position formerly occupied by the stalk as it traversed the sphenoid. The site of the attachment of the stalk to the rostral division of the anterior lobe persists at least until 16 days.

The neural lobe. At 7 days of incubation the neural lobe is well formed and contains a large cavity continuous with the third ventricle of the brain (fig. 1). The distal part of the lobe is wide from side to side and is attached to the infundibulum by a narrow neural stalk. This distal part, or neural lobe proper, becomes quite irregular in external contour due to numerous protuberances. Internally these contain evaginations of the cavity (figs. 10 and 11).

In later stages (18 days, fig. 11) the neural lobe is separated from the anterior lobe by a fairly thick layer of connective tissue (fig. 11). While apparently this is not so thick as in the whale (Wislocki and Geiling, '36) it is still sufficient effectively to separate the two lobes and thus prevent the intimate

contact of neural and intermediate lobes usually seen in vertebrates.

DISCUSSION

The partial division of the anterior lobe into two parts by a groove which lodges the internal carotid arteries, and a massive anastomosis between them, is a matter of some interest. The condition is illustrated by Rossi (1896) but without particular comment, and is well described and pictured for early stages by Lups ('29).

The determination of whether these two divisions represent fundamentally different lobes of the hypophysis will require more detailed studies upon younger embryos. The possibility must be borne in mind however that the caudal division may represent the lobo medio of Rossi. Or, if one follows the terminology of Woerdemann ('14) and Lups ('29), the caudal division might be thought of as derived from Rathke's pouch proper while the rostral division would be a composite of Mittelraum, Vorraum and Ventralsäckchen, to the first of which the Lobuli laterales, or tuberal processes, find attachment (compare Lups, fig. 9).

By employing careful cytological fixation and staining methods Rahn ('38) has found differences in the cells of these two divisions. According to him the caudal part represents typical anterior lobe tissue; the cephalic (or rostral) part "is chiefly characterized by an exceedingly faint-staining acidophile of different color and finer granulation."

In referring to the pars intermedia of the hypophysis Stendell ('14) remarks, "Bei keiner Klasse der Vertebraten ist der Zwischenlappen so winzig wie bei den Vögeln." He states however that a pars intermedia has been found in all birds studied including with others the domestic fowl, the duck and the goose. In his figure 48, accredited to Gentes, a sagittal section of the hypophysis and infundibulum of *Anas boschas* is represented, which shows a persistent residual lumen and a thin layer in contact with the neural lobe which is labelled the pars intermedia (Zwischenlappen).

DeLawder, Tarr and Geiling ('34) state that "a minute area of epithelial cells corresponding to the pars intermedia was found in the posterior pole of the pars anterior in serial sections of one chicken hypophysis." Rahn ('38) cautiously records an apparently similar observation: "Morphologically there is no evidence for a pars intermedia, though in a corresponding region the cytology reveals a few cell cords which can be variously interpreted."

The fact that no discrete pars intermedia can be pointed out in the chick during the last 2 weeks of incubation as found in the present study does not preclude the possibility that a pars intermedia may differentiate in situ at some later time. It is reasonable to suppose that a pars intermedia, if it should differentiate later, or scattered cells which may have the function of this lobe, would be located in what has been called the caudal division of the anterior lobe, in spite of the statement of DeLawder, Tarr and Geiling that "the principle which causes dilation of the melanophores of frogs seems to be concentrated in the anterior end of the pars anterior." Experiments are now under way to test the validity of this assumption.

That the pars intermedia and the neural lobe can perform at least some of their functions independently now seems clearly indicated. The studies of Geiling and associates on the chick and the whale, where neural and buccal lobes are separated in the adult, show that vasopressor, oxytocic and antidiuretic activity is exhibited by extracts of the neural lobe alone, while extracts of the buccal lobe alone cause marked dispersion of the melanophores of the frog.

Evidence of a similar kind has been obtained by the writer (Atwell, '35, '37) in transplantation experiments with two species of frogs. The epithelial hypophysis, independent of neural tissue, when transplanted into the vicinity of the otic vesicle, at the tail-bud stage or slightly later, develops and functions to maintain the dark color of the tadpole, without having made any contact with the brain.

SUMMARY

The morphogenesis of the hypophysis cerebri of the domestic fowl has been studied by means of serial sagittal and frontal sections taken at the following stages of development: 7, 9, 11, 13, 15, 16 and 18 days of incubation and 1 day after hatching. Wax-plate reconstructions were made from sagittal sections of the 7-, 11-, 15- and 18-day and the day-old chicks.

Attention is called to the fact that the anterior lobe is partially subdivided into rostral and caudal divisions by a groove which lodges the internal carotid arteries and a large anastomosis between them. The possible phylogenetic significance of these divisions is discussed briefly.

The hypophysial cavity disappears after the ninth day of incubation in the chick and so it is impossible to point out a discrete *pars intermedia*. Except for certain transient contacts found at about 11 days of incubation the neural and buccal lobes are not in intimate contact. At 18 days a moderately thick layer of connective tissue separates the two lobes.

The two halves of the *pars tuberalis* remain separate until relatively late stages. They are found to be meeting and fusing in the midline both rostral and caudal to the infundibulum in the day-old chick.

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PLATE 1

EXPLANATION OF FIGURES

Figures 12 to 14. Wax-plate reconstructions of the epithelial hypophysis of chick embryos, $\times 50$. Abbreviations: R.D., Ant.L., rostral division of the anterior lobe; C.D., Ant.L., caudal division of the anterior lobe; Gr., groove which lodges internal carotid artery and partially separates rostral and caudal divisions. T.P., tuberal processes; P.T., pars tuberalis; Ep.St., epithelial stalk.

12 Epithelial hypophysis of a chick of 7 days of incubation, viewed from the left side.

13 Epithelial hypophysis, chick incubated 11 days, viewed from the left and behind.

14 Epithelial hypophysis of a chick incubated 15 days, viewed from the left rostro-dorsal aspect.

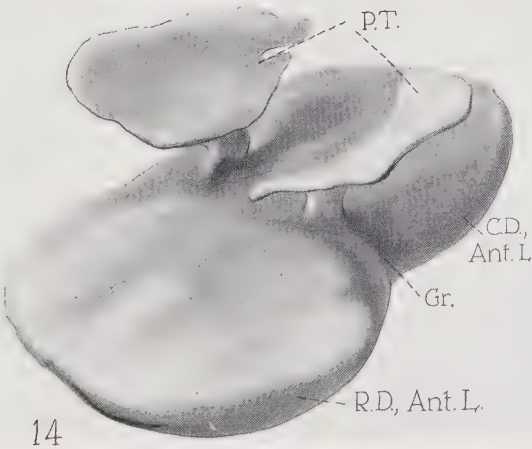
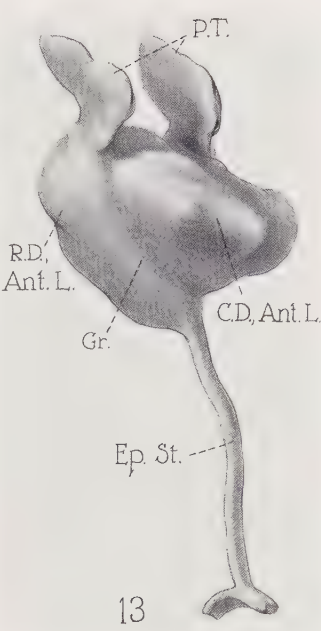
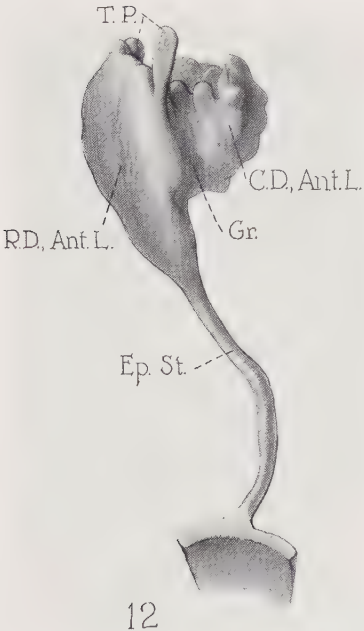


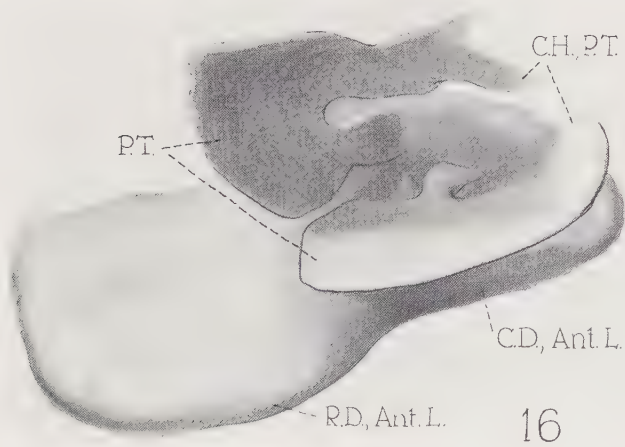
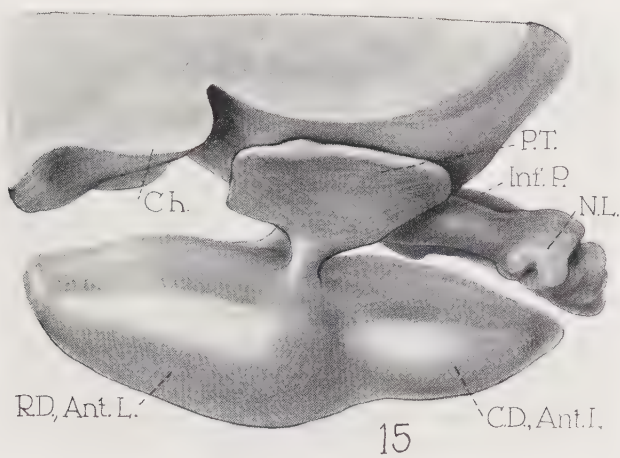
PLATE 2

EXPLANATION OF FIGURES

Figures 15 and 16. Wax-plate reconstructions of the hypophysis of chick embryos. $\times 50$. Abbreviations: Ch., optic chiasm; R.D., Ant.L., rostral division of anterior lobe; C.D., Ant.L., caudal division of anterior lobe; P.T., pars tuberalis; C.H., P.T., caudal horns of pars tuberalis; Inf.P., infundibular process; N.L., neural lobe.

15 Hypophysis and adjacent brain wall from chick incubated 15 days, viewed from the left side.

16 Epithelial hypophysis of a chick incubated 18 days, viewed from the left and above.



THE EFFECTS OF CRYSTALLINE SEX HORMONES ON SEX DIFFERENTIATION IN AMBLYSTOMA

II. TESTOSTERONE PROPIONATE

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THREE PLATES (TWELVE FIGURES)

INTRODUCTION

It has been reported recently (Burns, '38) that repeated injections of small doses of crystalline estrone (oil solution) into larval *Amblystoma punctatum* bring about progressive transformation of the testes of genetic males most of which, by the time metamorphosis has been completed, come to resemble closely ovaries of an earlier period of differentiation. From the less completely transformed cases, including a very small number in which reversal is only beginning, it can be seen that the histological stages of transformation are identical with those established for parabiosis experiments and grafting of the embryonic gonad primordium. In each case the process takes the form of a gradual change in sex type of the differentiating rete apparatus, which has the effect of reversing the relative proportions of cortex and medulla in the differentiating gonad. The differentiation of ovaries is not significantly changed by the estrogen.

While the action of estrone on the differentiating gonads is striking, the developing gonoducts are not so markedly affected. However, the oviducts (mullerian ducts) are definitely enlarged in diameter and always extend farther posteriorly than the best developed control ducts. These changes occur alike in larvae of either sex. The mesonephric, or wolffian ducts are apparently unaffected. Finally, the cloacae

are unchanged grossly, retaining the undeveloped larval condition (a histological study of the cloacal glands has not been completed). These results are briefly recapitulated for comparison with the present experiment, and to emphasize the apparently specific nature of the effects obtained. The development of female characters is favored by estrone; testes are transformed into ovaries, the developing oviducts are stimulated in both sexes, while male accessory structures (wolffian ducts and cloaca) appear to be unaffected rather than inhibited.

THE EXPERIMENT

In this experiment the subjects were twelve larvae of *A. punctatum* (Linn.) from the Rochester area, about 60 days of age, together with ten specimens carried as controls. Due to slow rearing at a relatively low temperature, they were only 24 to 28 mm. in length at the beginning of the experiment; but because of the comparatively advanced age, were probably undergoing early differentiation at this time.

Each experimental animal received a total of 250 γ of crystalline testosterone propionate¹ (dissolved in oil) administered in ten doses distributed over a period of 45 days. Injections therefore came at intervals of 4 to 5 days. Each consisted of 0.005 cc. of oil containing 25 γ of the crystalline substance. The control group received injections of oil only for the duration of the experiment. Injections were made into the body cavity using an improvised micro-injector, with the animals under light anesthesia.

One experimental animal died early and was discarded. Throughout the period covered by the experiment growth was slow but uniform in both experimental and control groups, and at the end the subjects approximated 40 mm. in length. The experiment was terminated as metamorphosis was beginning in controls, when a rapid and extreme enlargement of the cloaca in every experimental animal indicated the

¹ For the hormone used in this experiment we are indebted to the courtesy of Dr. Erwin Schwenk, of the Schering Corporation.

activity of the hormone. The cloacal response was so marked that an examination of the internal structures seemed called for, while the number of experimental animals made it inadvisable to divide the group in order to prolong the experiment. The essential data are presented in table 1. As compared with the earlier work with estrone, the experiment with testosterone propionate terminated prior to metamorphosis, and involved larvae which were somewhat less developed at the beginning of the experiment.

TABLE 1
Testosterone propionate administration

DATE OF INJECTION	AGE	LENGTH	DOSE	NUMBER OF CASES	COMMENT
	<i>days</i>	<i>mm.</i>	<i>γ</i>		
June 23	60	24-28	25	12	1 died
30			25	11	
July 5			25		
10		28-30	25		
15			25		
20			25		
24		33-35	25		
28			25		
August 1			25		
5			25	11	Approaching metamorphosis
7	105	40	Total 250		Preserved. Duration, 45 days

RESULTS OF THE ADMINISTRATION OF TESTOSTERONE PROPIONATE

1. *Gross examination*

During the last 2 weeks of the experimental period, growth of the cloaca became conspicuous in all injected animals. At the end swelling was extreme with eversion of the cloacal lining (fig. 2), exposing the prominent rugae on which the cloacal glands have their openings. This appearance was in extreme contrast to the cloacae of controls, which were entirely undeveloped and indifferent as to sex (fig. 1).

Removal of the ventral body wall and gastro-intestinal tract exposes the urinogenital organs, as in figures 1 and 2. In controls of either sex (fig. 1) a slender, compact, cord-like structure extends along the lateral border of each kidney,

terminating posteriorly in the cloaca. Anteriorly these cords reach the limits of the body cavity at the pronephric level, just behind the pericardium. During early development they contain the primary kidney duct (pronephric duct), enclosed in a sheath of connective tissue and partially invested by a fold of peritoneum. Subsequently this duct becomes the mesonephric duct. Later the developing oviduct or mullerian duct originates anteriorly and grows backward lateral to the mesonephric duct. The cord then contains both gonoducts, although the oviduct is not complete for some time. The thickness of the cords in the posterior region of the body is made clearer in the male specimen of figure 1, by removal of small blocks of kidney tissue. In normal larvae at the end of the period covered by the experiment, the posterior tips of the growing oviducts are found histologically at a level near the anterior end of the kidney, or occasionally as far back as the anterior pole of the gonad. The level is subject to considerable individual variation, and to marked lateral inequalities as well. The variations noted, however, appear to be independent of sex at this stage, although the occurrence of the longest pair of oviducts noted in a female with well-developed ovaries, may indicate that the beginning of sex dimorphism is at hand.

It is established, therefore, that the cords of control larvae contain, in the region anterior to the kidneys, both the developing oviducts and the slowly retrogressing anterior portions of the mesonephric ducts (the original pronephric segment of these ducts). The development attained by the oviducts in controls of this experiment is on the whole slightly less than that described for the estrone series, due to a corresponding difference in age.

When the experimental animals are compared with this picture of normal development, enormous difference appears (fig. 2). Instead of the slender cord-like fold of control specimens, a massive structure is seen, frequently convoluted, with a diameter equal to the width of the entire kidney.

Anterior to the kidneys the cord shows less hypertrophy and is usually straight, still there is a great enlargement over the normal cord in this region. While the general impression from gross examination would assign the hypertrophy to enlargement of the wolffian duct (a view later confirmed by histological study), it was not possible by macroscopic means to determine the condition of the oviduct, or to what extent it contributed to the size of the whole cord.

The gonads of all experimental animals (fig. 2) were rather similar externally and of a nondescript character, so much so that it was not possible to distinguish sex on the usual grounds. None approached even remotely the development of normal ovaries. Most were fairly close approximations to testes in their external appearance; however, past experience with intersexual gonads in *Amblystoma* has shown that atypical gonads of this category can only be classified by study of serial sections.

2. Histological examination. The gonads

Identification of sex by histological study was not difficult. In general, histological reversal of sex type in the gonad is not so thoroughgoing as was reported for estrone. Four of the eleven experimental larvae possessed gonads which conformed so closely to normal testes (as shown in fig. 4) that there was no hesitation in accepting these cases as genetic males. The treatment apparently had failed to accelerate development beyond normal limits. In this respect the findings are again in agreement with the results of administering estrone, and it appears that in both sexes of *Amblystoma punctatum*, during the larval period, administration of a homotypic hormone does not accelerate gonad differentiation beyond normal, at least with the dosages employed. This conclusion appears to hold good only for the gonads and not for accessory structures.

The remaining seven experimental larvae proved to be females exhibiting different degrees of response to the androgen. Responses ranged from cortical inhibition with

reduction and consolidation of the ovarian sacs, through various stages of medullary growth with persistent cortex, ending with almost complete suppression of cortex. These different stages of histological transformation to some extent characterize individual cases, but are also found in different regions of the gonads of the same individual, as will appear in cases to be described.

An example of cortical inhibition with little evidence of positive transformation, appears in the gonad at the right in figure 5, which is merely a strongly inhibited, abnormal ovary. Compare this gonad with the control ovaries of figure 3. The ovarian sac of the experimental gonad has undergone reduction and the ovocytes of the cortex have been arrested in their development on entering the growth phase. Most of these ovocytes are now pycnotic. Other regions of this gonad are similar but in some places medullary development was under way in the hilar region to a degree like that seen in figure 6 (from another specimen). However, the case under consideration illustrates particularly well the different stages of reversal frequently encountered in one individual. In the same subject the other gonad was, in most of its extent, completely consolidated, closely approaching the structure of a testis (fig. 5, left). Proliferation of stromal cells derived from the rete cords has completely obliterated the ovarian sac, and only locally does this gonad resemble an ovary.

Other cases show a more advanced stage of reversal and also greater uniformity in all regions of the gonads. An example is shown in figure 6. The gonad at the left is an ovotestis in which the cortical component (seen distally) is often as large as the medullary mass at the hilus; but always appears degenerate or necrotic. The condition pictured is found throughout the entire organ. The gonad of the opposite side was also usually of this character, but frequently, as in the section pictured (fig. 6, right), the cortical component had been eliminated, leaving only a vestige in the form of the small tag or lappet seen at the distal margin. In such localities this gonad can only be called a testis.

In summing up we may say that, in the developing gonad, testosterone propionate causes inhibition of cortical development in ovaries, with obliteration of the ovarian sac, followed by medullary development and progressive elimination of cortex. In many gonads these changes lead locally to a rather close approximation of the normal testis. In no case, however, has the hormone effected a complete reversal of sex type in the gonad, being clearly less efficient in this respect than estrone. The difference is doubtless due in part to the embryological status of differentiating ovaries, where the rete elements, on which male differentiation depends, become greatly reduced at an early stage. The incomplete reversal of the gonads is in sharp contrast with the high degree of development induced precociously in the duct system and cloaca.

The gonoducts

The appearance of the gonoducts after dissection has already been described. Sections through the cords containing the ducts in control specimens, are shown in figures 7 and 8, near the anterior limits of the body cavity and through the anterior end of the mesonephros, respectively. The latter section is not far from the termination of the mullerian ducts in the specimen in question, and represents an average condition. In animals just beyond metamorphosis (therefore but slightly older than the present group) appearances are practically the same, and for other views showing the normal condition of the ducts see Burns ('38) figures 4 and 11.

Figure 7 is actually from a control of the older group but is typical in all respects of this experiment. The mullerian ducts are placed ventrolaterally and consist of epithelial cords, usually lacking a distinct lumen, and closely invested by a mesenchymatous adventitium. The wolffian ducts are even more rudimentary in this anterior region, in which they will soon disappear entirely. They are represented by slender epithelial cords, also usually without a lumen, composed of from three to five cells per cross section. At the level of the first mesonephric tubules (fig. 8) the size relations of the

ducts are reversed, since at this point the wolffian duct serves as mesonephric duct and is a functional and permanent structure in both sexes, later acquiring a special role as vas deferens in the male. These sections illustrate the average normal development of both ducts in the anterior half of the body cavity, at this stage. Farther posterior in the gonad region the mesonephric ducts are shown again for a normal female and male, respectively, in figures 3 and 4. In the first a suggestion of the growing tip of the oviduct is seen at the right, in the form of a few loosely aggregated cells occupying a fold of the peritoneum along the lateral surface of the mesonephric duct. In the male specimen of figure 4 no signs of the oviduct are present and the mesonephric ducts are typical of control specimens. That at the left is seen at the point of entrance of the terminal segment of a mesonephric tubule. Note that this tubule is wide in diameter and enters the duct virtually in the plane of the section.

Figures 9 and 10 are views in experimental larvae, at approximately the same levels anterior to the kidney, for comparison with the normal ducts of figures 7 and 8. Figure 10 just precedes the position of first mesonephric tubules. A single pair of large ducts appear—the wolffian ducts; no histological traces of the oviducts can be found, and these structures have either failed to develop at all in experimental animals, or have subsequently degenerated, leaving no clear histological traces. This is true at all levels. The hypertrophied mesonephric ducts also appear in figures 5, 6 and 11, in other experimental larvae, taken respectively at the middle gonad region, near the posterior poles of the gonads, and a short distance behind the gonads. By referring again to figure 2 it is seen that only the last of these sections is through the thickest parts of the mesonephric ducts, the region of greatest hypertrophy lying between the posterior ends of the gonads and the cloaca.

For convenience in considering the effects of treatment on the mesonephric ducts, we may divide them into three regions. An anterior segment, extending from the pronephric

level back to the first mesonephric tubules, is destined in normal development to disappear entirely. Under the influence of the androgen this region of the primitive duct persists, undergoes great hypertrophy, and acquires the histological character of a vas deferens; although it can never, by virtue of its anatomical position, bear such relationship to the gonad. Although hypertrophied this region is more slender than the rest of the duct and does not show convolutions. The second region lies opposite the gonads and is associated with the 'sexual kidney.' In all experimental larvae it becomes much heavier than the anterior segment and is the first portion to develop convolutions, although this condition has not been attained in all cases. The level from the gonads to the cloacal wall becomes largest of all in point of diameter, but in most cases remains unconvoluted.

Histologically the hypertrophy consists of three elements—proliferation and differentiation of the mucosa, a thickening and condensation of connective tissue to form a dense adventitial sheath for the duct, and a marked dilatation of the tube. These processes, however, are not represented to the same extent in the three delimited regions. In the anterior region where the duct is least hypertrophied, the epithelium has thickened and differentiated but the lumen is relatively small (fig. 9). The sheath on the other hand is usually thick and dense, with a lamellar arrangement of cells, suggestive of the early differentiation of smooth muscle. In the middle or gonad region of the duct, the lumen is larger, the mucosa taller and often folded considerably. Figure 5 suggests this condition very well but does not illustrate the maximum. The sheath here is also well developed but relatively less so than in the anterior region. The posterior segment is characteristically dilated (figs. 6 and 11). In consequence the lumen is large and the mucosa flattened or attenuated; the sheath is decidedly thinner and looser in structure.

The 'collecting tubules' or 'ureters' of the mesonephros

The true mesonephros, or 'sexual kidney,' is characterized by the presence of a single series of units consisting each of a nephrostomal canal, a renal corpuscle, and a highly tortuous tubule differentiated histologically into several regions (with the homologies of which we are not now concerned). Each tubule joins the mesonephric duct by an almost straight terminal segment. Although secondary and tertiary (etc.) secretory units are not present in the sexual region of the kidney, this terminal portion of the tubule corresponds morphologically to the collecting tubules of the metanephric region, and may be so designated. The tubules of the 'sexual kidney' are functional nephric units which in the male also acquire secondarily the function of sperm conductors, by virtue of connections established with the testis through the rete passages, vasa efferentia, and the longitudinal duct (of Bidder). Functionally and morphologically they correspond to the epididymal tubules of higher forms, but retain at the same time their renal functions; and while the convoluted tubule continues as an excretory structure the terminal portion becomes modified histologically with maturity, finally resembling a duct rather than a secretory tubule. This change in character is accompanied by an anatomical change in which the terminal limb passes obliquely backward to the point of entry into the mesonephric duct. These changes are of the same type but less extreme than those which occur in the collecting tubules of the metanephric region of males (see the recent description of Rodgers and Risley ('38) for *Amblystoma tigrinum*).

Such changes in the character of the collecting tubules occur prematurely in all the injected animals of this experiment, without reference to sex. In controls of both sexes the collecting tubules pass from the dorsal part of kidney in an almost straight course ventrolaterally, bending slightly beneath the cardinal vein, to reach the medial surface of the mesonephric duct. So nearly transverse is the plane of this

passage that the entire course is frequently cut in a single section of 10μ and rarely are more than two or three sections traversed (fig. 4, left). In experimental animals the tubules are histologically altered and greatly constricted, so that they often appear almost to lack a lumen (fig. 5, right). The tubule is often slightly tortuous, and passes obliquely backward over varying distances before joining the mesonephric duct. Such tubules are seen in figure 11, right, where, due to their oblique course two are seen in the same section. After reaching a position in the supporting fold adjacent to the duct, collecting tubules, in several cases, traversed from fifteen to forty sections in a posterior direction before entering the duct. The tubules of the metanephric kidney are much more affected, but a complete study of the metanephric and cloacal regions has not yet been made.

The tubular outlets of the testis

Under this head are included the vasa efferentia and the longitudinal duct (Bidder's duct) of the testis. The vasa efferentia arise by a tubulation of the rete cords, which grow from the mesonephros into the developing genital ridges at the beginning of differentiation, in the form of loose cellular strands. During larval life they retain this character, gradually becoming more compact in the male and more diffuse and rudimentary in the female. It is not until well beyond metamorphosis that the cords acquire a tubular form and eventually, as maturity approaches, become patent ducts. At the same time, the longitudinal duct arises by the establishment of a number of somewhat irregular cord-like anastomoses between adjacent rete cords at their points of connection with the glomerular capsules. At times these anastomoses apparently connect capsule with capsule, and the future duct virtually lies within the confines of the mesonephros. Later the duct shifts its position to lie more within the mesorchium, but always in close association with the ventromedial surface of the kidney.

In many of the experimental animals, and especially in the males, local segments of the longitudinal duct are well differentiated before metamorphosis. The type of structure encountered is shown in figure 12. Rarely are more than two or three consecutive segments of a duct differentiated to this extent, and in no case was a complete duct found. The independent appearance of isolated portions of the duct is not odd, however, in view of its origin from local links, as described.

In the case of the longitudinal ducts, there is no doubt that, in normal development, potential sexual dimorphism is established early with respect to the rudiments necessary for their formation; for they are almost without exception better developed in the males of the experimental groups. Very definite rudiments appear locally in some females, however, which clearly exceed the best development found in any normal at metamorphosis, where, indeed, they are usually difficult to trace with certainty.

In late larval life the efferent ducts of the testis normally show marked sexual dimorphism. As we have seen, the rete cords which give rise to the efferent ducts are invariably more robust in males, and exceedingly ill-defined in females. In this experiment, however, even the male rete cords do not respond to experimental treatment as do the wolffian ducts and collecting ducts, or even local segments of the longitudinal duct. The reason for this is not entirely clear, and may be connected in a manner now obscure with the fact already established—that the testis itself is not accelerated in its development by a homotypic hormone. Doubtless an age factor must be taken into account also, and in postmetamorphic life a better response on the part of these ducts is probable.

The failure of the efferent ducts to develop in females is more easily understood, for only in the earliest stages of differentiation are the cords of the rete apparatus well formed. Early in ovarian development the parts of the cords within the gonad give rise to the ovarian sacs, the walls of which

become greatly expanded and attenuated. The proximal parts of the cord, within the mesovarium, retrogress rapidly. It is clear that the rudimentary parts necessary for the formation of vasa efferentia and longitudinal ducts, while initially present in the female, are transient, and afford a comparatively poor embryological foundation for male differentiation later on.

It appears, then, that in the diverse series of links that eventually give rise to the male duct system, not all the elements respond in the same degree to hormonal stimulation. The actual response elicited is to be explained only in part on the basis of embryological representation. An age factor, and perhaps a difference of threshold correlated with it, must also be considered.

The cloacae

A final histological study of the changes in the cloacae has not been made. Externally the enlargement is enormous and, while there is variation in individuals, the phenomenon is not sex restricted so far as its gross aspects are concerned (fig. 2). Some of the largest examples belong to genetic females. It may be, however, that when the separate glands are compared histologically, distinct sex differences will appear.

A preliminary histological examination shows at once that, although the glands are greatly proliferated, the massive enlargement of the cloaca is not due to glandular development alone. Much of the swelling is attributable to a dense intertubular connective tissue of mucoid type, universally present in experimental animals. To this tissue as much as to the growth of the glands themselves, is due the extreme external enlargement. There is also a thickening and folding of the cloacal mucosa to form heavy rugae, visible whenever the lining has been everted, as in some specimens shown in figure 2. The glands of control cloacae are extremely rudimentary, and the cloacal lining thin and smooth.

SUMMARY AND CONCLUSION

1. The synthetic androgen testosterone propionate was administered to larval *Amblystoma punctatum*, in the form of repeated small doses over a period of 45 days, terminating at the first indications of metamorphosis. During the period covered by the experiment the gonads of normal larvae become well differentiated, while the gonoducts and cloacae are quite immature and sexually indifferent. The derivatives of the rete apparatus show a sexual dimorphism in accordance with the sex type of the gonad.

2. In experimental larvae the hormone produces all degrees of intersexuality in the developing ovary; the development of testes is not affected by a homotypic hormone. The process of sex reversal in differentiating ovaries is like that previously described, following parabiosis and embryonic grafting of the gonad.

3. The hormone induces marked hypertrophy and sexual differentiation in the mesonephric or wolffian ducts of both sexes, and the 'collecting ducts' of the sexual kidney are modified. The development of the rudimentary oviducts, on the contrary, is completely inhibited. In the latter respect the androgen differs from estrone, which has no effect upon the differentiation of the male ducts.

4. The cloaca and its glandular derivatives are also greatly hypertrophied in experimental larvae of both sexes, while the cloacae of controls are completely undifferentiated. The individual cloacal glands have not been fully studied as yet.

5. The tubular derivatives of the rete apparatus—the vasa efferentia—for reasons not fully appreciated, fail to respond to the hormone, resembling the testis itself, in this respect; but there is premature differentiation of the longitudinal duct in some individuals of both sexes.

6. So far as this study goes, testosterone propionate reacts specifically with the developing reproductive system of *Amblystoma*. While the differentiation of the testis is not accelerated beyond the normal, male accessory structures are precociously developed. On the other hand, the development

of the ovary and oviduct is inhibited. Thus far no evidence for the frequently reported estrogenic action of male hormones has been encountered. The conclusions advanced here apply, of course, only to the larval period of *Amblystoma*, under the conditions described.

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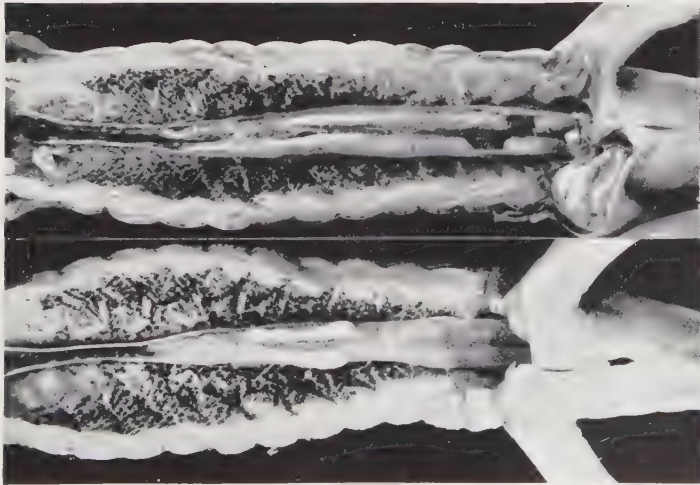
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PLATE 1

EXPLANATION OF FIGURES

1 Typical female and male larvae of *A. punctatum*, showing development of the urinogenital system just before metamorphosis. The slender white cords lying lateral to the kidneys and extending to the anterior limits of the body cavity contain the developing gonoducts. The male specimen has blocks of kidney tissue removed to show the thickness of the mesonephric ducts posteriorly. The oviducts at this stage do not extend much behind the anterior ends of the mesonephroi. Note that the cloacae are quite undeveloped, the lips and surfaces lying flush with the body wall. $\times 5\frac{1}{2}$.

2 Four experimental larvae of the same age, showing hypertrophy of the mesonephric (wolffian) ducts and cloacae in individuals of both sexes. The case at the left is one of the most modified, that at the right the least modified in the entire experiment. The portion of the duct lying anterior to the mesonephros is also hypertrophied, although this part would shortly disappear entirely, in normal development. The gonads are more or less indeterminate, with no normal ovaries appearing. $\times 5\frac{1}{2}$.



1 Normal



2 Experimental group

PLATE 2

EXPLANATION OF FIGURES

3 A section through the middle gonad region of a normal female, showing typical ovarian structure. A mesonephric duct appears at the upper right, while a few loosely aggregated cells, in a fold of the peritoneum, mark the point of appearance of the rudimentary oviduct. $\times 130$.

4 Corresponding section in a male larva. The mesonephric ducts are like that of the female just pictured. The duct at the left is receiving the terminal limb of a mesonephric tubule. The oviduct has not reached this level in this specimen. $\times 130$.

5 An experimental larva showing a high degree of modification of the gonad at the left, which approximates a normal testis; the other remains a greatly inhibited ovary. The hypertrophied mesonephric duct appears at the upper right. $\times 130$.

6 Another experimental larva, showing the posterior gonad region. The gonad at the left is an ovotestis, in which the testis-like medulla (proximal) is developing at the expense of an atrophic cortex. In the other gonad only a vestigial flap of the cortex remains. The mesonephric duct (left) is widely distended at this level. $\times 130$.

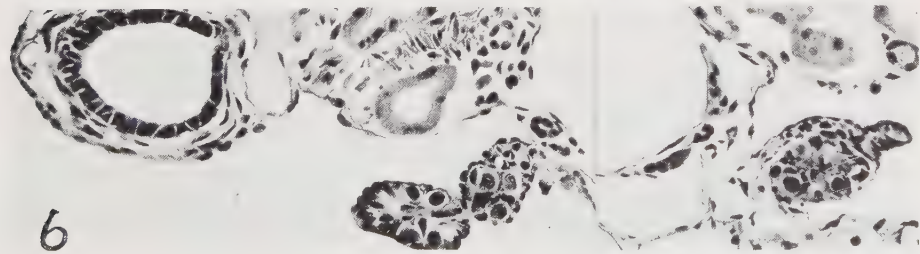
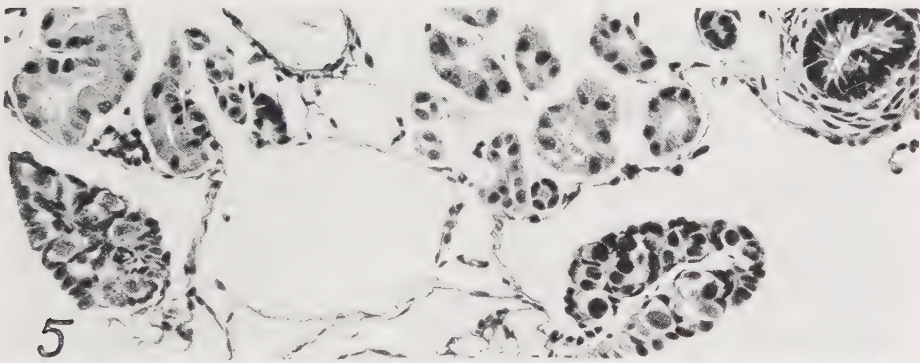
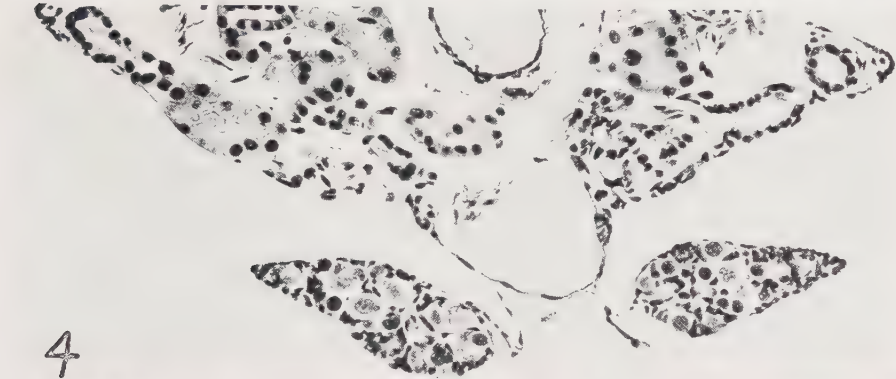
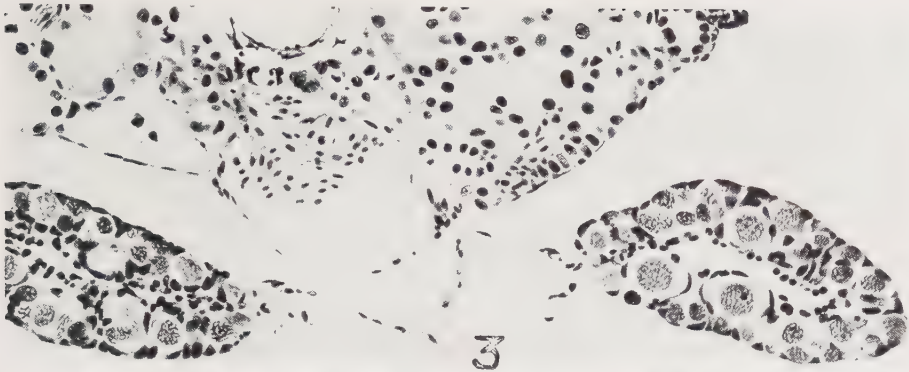


PLATE 3

EXPLANATION OF FIGURES

7 The cords containing the gonoducts in a normal larva (fig. 1) cut in the anterior body cavity. The rudimentary oviducts lie ventrolaterally, the wolffian ducts (retrogressing) are dorsomedial in position. $\times 130$.

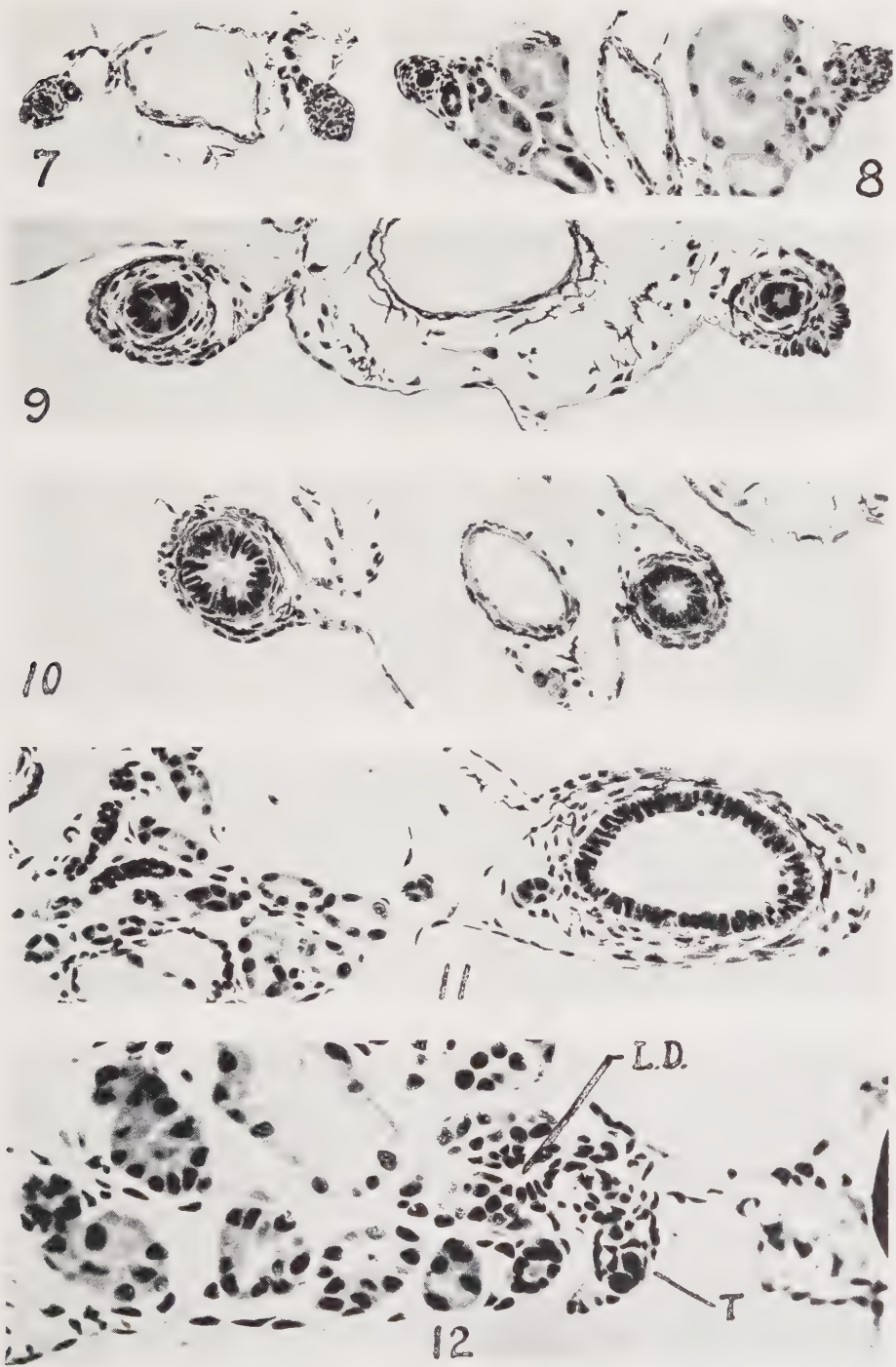
8 The gonoducts seen farther back, at the anterior end of the mesonephros. The oviducts are less developed at this level, the mesonephric ducts are functional and permanent structures here. $\times 130$.

9 A level comparable with figure 7, from an experimental case. The single greatly hypertrophied duct on either side is the wolffian duct. No traces of the oviducts are to be seen. $\times 130$.

10 Another specimen, cut near the anterior ends of the mesonephroi, therefore comparable to figure 8. The oviducts are not present. $\times 130$.

11 View just posterior to the gonads, showing the distended mesonephric duct and two collecting ducts passing through the supporting fold to join it. Due to the oblique backward course of these ducts two and sometimes three may appear in a single transverse section. $\times 130$.

12 View at the posterior pole of the testis, T, showing the differentiation of the longitudinal duct (of Bidder), L.D., in this region of an experimental male larva. $\times 200$.



REGENERATION OF THE LIVER IN HYPOPHY- SECTOMIZED WHITE RATS

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TWO FIGURES

Rapid regeneration of the liver occurs in the residual lobes following partial hepatectomy. This has been repeatedly demonstrated in animals. For a résumé of the earlier work on extent of regeneration and on the factors which regulate regeneration the reader is referred to the excellent paper by Brues, Drury and Brues ('36).

After the removal of about 70% of the liver, the residual lobes soon hypertrophy so that in 24 hours there is about a 50% increase in their total weight. But during this first day there is no increase in the number of hepatic cells. Mitoses begin to appear at the end of the first day and during the second and third days after operation the number of hepatic cells may increase 65%. In 2 weeks an amount of hepatic tissue equal to or greater than the size of the original liver has been restored.

Factors which control this rapid growth of hepatic tissue are not understood. Regeneration does not appear to depend entirely on the requirements of the organism for hepatic tissue, for when portal blood is diverted from the liver through a fistula into the vena cava the amount of regeneration following partial hepatectomy in dogs is very small (Mann, Fishback, Gay and Green, '31). Likewise when the portal vein in white rats is partially occluded at the time the liver lobes are removed the amount of tissue which regenerates in the residual lobes is greatly reduced (Stephenson,

'32). On the other hand when the amount of portal blood reaching the liver lobes after partial hepatectomy is increased, as is possible in chickens (Higgins, Mann and Priestley, '32), the amount of liver that regenerates is definitely increased. Regeneration of liver therefore would seem to be related to the amount of blood delivered to it through the portal vein. Diet also is a factor in regeneration of the liver. Restoration, following central necrosis induced by chloroform, is more extensive when the animals are fed a protein or a carbohydrate diet than when fed fat (Davis and Whipple, '21).

Since the growth of the residual lobes, following partial hepatectomy, is so rapid we believed it essential to investigate the relationship of the pituitary gland to hepatic regeneration. As is well known, regulation of growth is considered to be a function of the growth hormone elaborated by the pituitary body.

This report covers the result of a study in which we have followed the extent of hepatic regeneration in animals from which the pituitary gland had been removed. Hypophysectomy induces in white rats a number of changes, among which are loss of appetite with consequent loss of body weight. Since diet in itself has a definite relation to the amount of liver which will regenerate, it was necessary to control the food factor. Consequently the extent of hepatic regeneration following partial hepatectomy has been followed in three series of white rats: a) partially hepatectomized normal animals which ate adequate amounts of food, b) partially hepatectomized previously hypophysectomized animals and c) partially hepatectomized normal animals which were fed an amount of food daily, comparable in weight to that consumed by hypophysectomized animals.

METHODS

Male white rats, in good physical condition, weighing 180 gm. were used. Partial hepatectomy was done through a median abdominal incision extending posteriorly from the

xiphoid a distance of 4 cm. About 70% of the liver was removed. Ether anesthesia and satisfactory technic were employed. Hypophysectomy was performed through the parapharyngeal route, using ether anesthesia. Animals used for the study of hepatic regeneration in the absence of the pituitary were hypophysectomized 1 week before the liver lobes were removed. All animals were fed our regular laboratory ration. Through experience we had learned that hypophysectomized rats eat about 6 gm. of food daily. This is considerably less than half the amount of food a normal rat, weighing 180 gm., ordinarily consumes. Thus animals used for the study of the effects of a restricted diet on hepatic regeneration were provided 6 gm. of food daily for 1 week before the liver lobes were removed. After the operation the same amount of food was provided each animal daily for the period of observation.

Fifty-five rats were used to determine the effect on the weights of the livers of white rats of a) feeding adequate amounts of food, b) removal of the pituitary gland and c) feeding restricted amounts of food. One hundred and ten rats were subjected to partial hepatectomy and used in our study of hepatic regeneration under these same conditions. Of these, ten normal rats were killed to determine the average weight of the residual lobes of liver which remained after removing the portions excised during partial hepatectomy. Five rats which had been hypophysectomized for 1 week and five rats which had eaten the lesser amounts of food daily for 1 week were killed and the weights of the remaining lobes for these groups were determined.

Forty rats which had been maintained on their usual amounts of food were partially hepatectomized and the remaining lobes of liver were allowed to regenerate. Five of these were killed at 6 hours after operation, five each at 12, 18 and 24 hours after operation, and five at 2 days, 3 days, 1 week and 2 weeks after operation. These rats were weighed, lightly anesthetized and exsanguinated and their regenerating livers were removed and weighed.

Twenty-five rats weighing 180 gm. each were hypophysectomized and 1 week later they were partially hepatectomized. Five of these were weighed and killed 24 hours after partial hepatectomy, five more at 48 hours, five at 72 hours, five at 1 week and five at 2 weeks following the removal of part of their livers. The regenerating livers were quickly removed and weighed.

Twenty-five rats weighing 180 gm. each were given 6 gm. of food daily. After 1 week on this restricted diet they were anesthetized and the usual components of their livers were removed. After the operation they were restricted to 6 gm. of food each day. Five of these were weighed and killed at the same intervals of time following the operation as were the hypophysectomized-hepatectomized animals. The regenerating lobes of the liver were quickly removed and weighed.

One cubic centimeter of cortin was given daily in drinking water to each hypophysectomized rat. This was essential to prevent the adrenal insufficiency which ensues on loss of the adrenotropic hormone. Hypophysectomized animals not treated with cortin regularly succumbed to partial hepatectomy, whereas there was no mortality from partial hepatectomy among the hypophysectomized animals which were treated with cortin. Since the purpose of this inquiry was a study of the growth of a tissue in the absence of pituitary growth hormone the administration of cortin would in no way invalidate the results.

RESULTS

When the pituitary glands were removed from white rats characteristic systemic reactions ensued. Among these were anorexia and consequent loss of weight. During the first week following hypophysectomy the rats lost an average of 20% of their weight and in 4 weeks the average loss of weight was about 30% of the original weight. Coupled with this loss in weight of the body, the livers of these hypophysectomized animals were smaller. The average weight of the liver in normal rats of our strain weighing 180 gm. was

7.12 gm. One week after the removal of the pituitary from animals which weighed 180 gm. at the time of operation the average weight of the liver of five animals was 6.06 gm. After 4 weeks the average weight of the liver in hypophysectomized animals was 4.96 gm. Interestingly the percentage decrease in the weight of the liver was about equal to the percentage decrease in the weight of the body.

Animals which were maintained on a small amount of food (6 gm. daily) sustained a loss in weight of the body about comparable to the decrease in weight we had observed in

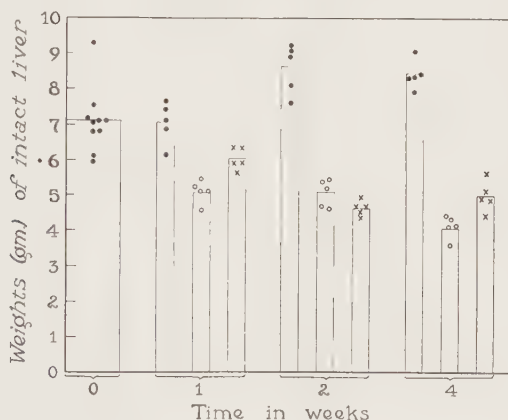


Fig. 1 Weights of livers in white rats maintained for 1, 2 and 4 weeks on a) normal diet (solid circles), b) restricted diet (clear circles); and c) after hypophysectomy (crosses).

hypophysectomized animals. The weight of the liver in animals after 1 week of the restricted food intake was considerably less than in those fed adequate amounts of food and even less than in hypophysectomized animals (fig. 1).

Accordingly, animals which were hypophysectomized and those which were fed the restricted diet had, at the time they were partially hepatectomized, smaller livers than the normal animals (fig. 2). Therefore the average weight of the residual lobes which remained after the operation was less than in animals which consumed the usual amounts of food.

The average weight of the residual lobes in five animals 1 week after hypophysectomy was 1.81 ± 0.06 gm., while in rats fed the reduced amount of food for a week these lobes weighed 1.56 ± 0.06 gm. In normal rats fed adequate portions of food this weight was 2.22 ± 0.04 gm. (table 1).

Twenty-four hours after the removal of a part of the liver the average weight of the residual lobes in control animals was 3.14 ± 0.13 gm. This is an increase in the weight of these lobes during that time of about 41%. In contrast to this the

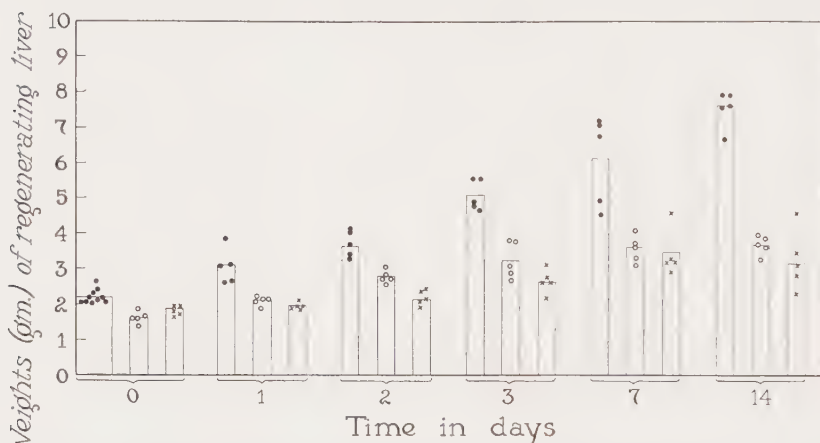


Fig. 2 Extent of hepatic regeneration in the three groups of animals, for 14 days following partial hepatectomy. (Circles and crosses as in fig. 1.)

increase in the weight of the residual lobes in hypophysectomized animals was but 7%, while in those fed the reduced amount of ration the increase in the weight of these lobes was 37%. The percentages of increase in the weight of the livers at 48 hours after operation were for the three groups (controls, hypophysectomized and controls fed restricted amounts of food) respectively 68, 22 and 78.

At 1 week after removing the portions of liver the average increase in the weights of the residual lobes was 3.89 gm. in the control group, 1.67 gm. in the hypophysectomized group and 2.02 gm. in the animals which were fed the lesser amounts of food. Two weeks after the removal of the liver, when the

experiment was terminated, the average increase in the weights of the regenerating livers was 5.38 gm. in the control group, 1.46 gm. in the hypophysectomized group and 2.16 gm. in those subsisting on the restricted amounts of food. Two weeks after partial hepatectomy the percentage increase in weight of the regenerating livers of normal animals equaled 242% of the amount left at operation, in hypophysectomized animals 81% and in animals on restricted diet 138%.

COMMENT

Rapid regeneration of the liver occurred in all animals which were fed an adequate amount of food. In animals to which the small amount of food (6 gm. per day) was given the amount of liver which regenerated in 2 weeks was 40% of that which regenerated in those animals which ate as much as they wished. In animals from which the pituitary gland had been removed the amount of liver which regenerated in 2 weeks was but 27% of the weight of the liver which regenerated in the adequately fed controls.

Although the amount of food which was consumed by the hypophysectomized animals was not regulated, estimates were made of the daily amount consumed by each animal and a comparable amount was fed to each animal of the restricted diet group. Two weeks after the removal of comparable portions of the livers from animals of these two groups (hypophysectomized and those fed the restricted amounts of food) the average weights of liver which had regenerated in the two groups were quite unlike. In animals from which the pituitary was removed an average weight of 1460 mg. of liver had regenerated by the fourteenth day, while in animals which were fed but 6 gm. of food daily, 2160 mg. of liver had regenerated during this same period. But during the same time an average weight of 5380 mg. of liver regenerated in each rat of the control group in which the amount of food eaten was not restricted.

Thus it seems evident that the amounts of food which were consumed by the animals determined largely the amounts of

liver which regenerated, but food may not be the only factor in the extent of hepatic regeneration in the hypophysectomized animals. Since an average increment of 700 mg. of liver regenerated in normal animals on the reduced diet above the amount which regenerated in hypophysectomized animals, the pituitary may play some role in controlling factors which regulate hepatic regeneration.

It must be observed, however, that regulation of the amount of food consumed will not provide a complete control, for it is well known that there are important differences between normal and hypophysectomized animals with regard to the extent of absorption and utilization of food. There are also large differences in the extent of voluntary muscular activities between these two groups of animals. Muscular activity is depressed far below normal in hypophysectomized rats and is greatly increased above normal levels in rats fed inadequate amounts. These factors of absorption and food utilization together with bodily activity may influence hepatic regeneration, so that too much significance should not be attached to the differences in the extent of regeneration between the hypophysectomized group and those on the restricted amount of food.

It is significant, however, that rapid growth of an organ will occur in the absence of the growth-regulating mechanism of the pituitary gland.

SUMMARY AND CONCLUSIONS

A study has been made of the regeneration of the liver in rats from which the pituitary gland had been removed. Since hypophysectomized rats develop an anorexia with loss in weight, it was necessary to observe regeneration in animals which were fed amounts of food equal to those ordinarily consumed by rats from which the pituitary gland had been removed.

Hepatic regeneration following partial removal of the liver has been studied in three groups: 1) normal animals which were fed adequate amounts of food, 2) animals from which

the pituitary gland had been removed 1 week before, 3) animals which were fed 6 gm. of food daily. Five animals in each group were killed at 24 hours, 48 hours, 72 hours, 1 week and 2 weeks following operation. The regenerating livers were quickly removed and weighed. The average weights of the two control groups were contrasted with the average weights of the test group at each of the intervals aforementioned. The following conclusions have been made:

1. Regeneration of the liver occurred in the hypophysectomized animals but to a far less extent than in normal animals.

2. Regeneration of the liver occurred in animals fed restricted amounts of food daily but likewise to a far less extent than in normals.

3. Greater amounts of liver regenerated in animals fed the restricted amounts of food than in the hypophysectomized animals which consumed about the same amount of food daily.

4. Regeneration of the liver seems to depend largely on the amount of food consumed; but on the basis of total average weights of liver which regenerated in the three groups of animals, it is possible that the loss of the pituitary gland may have had some inhibitory effect on the extent of regeneration.

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THE EFFECT OF FEEDING VENTRICULIN TO PREGNANT RATS, WITH SPECIAL REFERENCE TO THE SIZE OF THE RED BLOOD CELLS OF THE YOUNG

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ONE FIGURE

Red blood corpuscles in newborn animals are always larger than those in the adult. The number of red cells per cubic millimeter of blood is less than in the adult; so that a condition normally exists in fetuses, as well as in the young, which may be called a physiologic macrocytosis. As growth occurs, the red cells gradually increase in number per cubic centimeter and decrease in size until the adult condition is reached. In white rats the red cells of the newborn in dry smears measure approximately 10μ in diameter and have a mean corpuscular volume of about $150\mu^3$. When the animals are 70 to 80 days of age the average diameter of the red cells is slightly more than 6μ and the corpuscular volume is about $50\mu^3$. Wintrobe and Shumacker ('35) expressed the opinion that these changes which occur in the blood during normal development resemble the changes which occur in patients suffering from pernicious anemia who are subjected to some stimulus for blood formation.

Since it has been shown that pernicious anemia is the result of a deficiency in antianemic substances and may be relieved by the administration of these substances, Wintrobe and Shumacker have suggested that these antianemic principles may be similar to those factors which regulate the size and volume of the red cells during their normal development. The

macrocytosis which normally exists in the fetus and the newborn may be due to inadequate amounts of antianemic principle available for both the mother and the developing fetus. Presumably the fetus depends for these principles on the mother, for there is no evidence that the fetus elaborates its own antianemic substance. Consequently the maternal reserves may become so depleted during pregnancy that a macrocytic anemia often results.

The suggestion that the principle controlling the size of the red cells during development may be similar to the anti-anemic principle described by Castle ('29), led Stasney, Higgins and Mann ('37) to study the size of the red cells in rats born of mothers which had received gastric juice intraperitoneally during a part of the period of gestation. (In this procedure the amounts of the intrinsic factor available to the adult and to the fetus were considered to be increased.) They showed that when 1 cc. of human or swine gastric juice was injected daily during the last 12 days of pregnancy significant effects were induced in the blood of the fetuses. Blood of the newborn examined soon after birth contained more corpuscles per cubic millimeter than the blood of controls and the average size of the corpuscles was significantly smaller than that of the controls.

Since gastric juice, either human or hog, apparently contains some substance which stimulates the production of red cells and accelerates their maturation, as is indicated by a decrease in the size and volume of the corpuscles, we have fed ventriculin to pregnant rats during a part or all of the period of gestation and have studied the red corpuscles in the blood of the young rats soon after parturition.

Sturgis and Isaacs ('29) have demonstrated that there is an antianemic substance in stomach tissue and from their observations ventriculin has been evolved. Ventriculin is a dry granular product derived from whole stomach of the hog.

MATERIAL AND METHODS

Sixteen adult female rats of the Wistar strain were selected and bred. Two of these were placed on a diet containing ventriculin for the last 7 days of term; three of them were placed on the diet for the last 10 days of term, four for the last 14 days and seven for the entire term of pregnancy. The ventriculin diet consisted of our standard ration to which 20% by weight of ventriculin had been added. All rats ate freely of the diet. One hundred and twenty-five rats were born. Eighteen of these were born to adults which had been on the diet 7 days; nineteen were born to adults which had been on the diet 10 days; thirty-one to those on the diet 14 days and fifty-seven to those on the diet for the entire term of pregnancy.

At birth or as soon thereafter as possible, samples of blood were taken by cardiac puncture from each of the newborn rats. Using standard pipets the total number of red corpuscles per cubic millimeter was determined. The percentage of red cells of the whole blood was determined with hematocrit tubes, using heparin as an anticoagulant. Smears were made on clean glass slides and stained with Wright's stain. The blood smears were photographed on sensitized paper at 1000 magnifications and the paper was developed. The corpuscles were measured directly in microns from the images on the paper negatives. One hundred cells from each blood smear were measured.

The data assembled from the blood of 177 newborn rats representing twenty-five litters born to adults which had been fed the standard ration without ventriculin were contrasted with those derived from young rats born to mothers which had been fed the ration containing ventriculin.

RESULTS

The data assembled from these observations have been condensed into the accompanying tables. The results are in general agreement with those observed in young rats when gastric juice was administered to the mothers during pregnancy and show that the stomach preparation contains some

factor or factors which may accelerate the maturation of the erythrocyte during development.

There was considerable variation in the sizes of the red corpuscles in the blood of young rats born to normal adults fed our standard ration. The average diameter based on 17,700 measurements was $9.42\ \mu$, which was considerably less than the figure $10.09\ \mu$ reported in an earlier paper (Stasney, Higgins and Mann, '37) and based on 2000 measurements.

The average diameter of the red cells in animals born to mothers which ate the ration containing ventriculin on the last 7 days of pregnancy was not significantly less than the average diameter of the cells in smears made from the control animals. The average hematocrit value, however, was less and the average total number of cells per cubic millimeter was less than in the controls. The average diameters of the corpuscles in the blood of rats born to mothers which had eaten the ration containing the ventriculin for the longer periods (10 and 14 days, and for the entire term) were all lower than the control average. The range in the cell measurements, it will be observed in table 1, was large; yet the largest corpuscles encountered in any of the experimental groups were smaller than the largest observed in the smears made from blood of the control animals.

A comparison of the percentages of the various sizes of red corpuscles in the smears shows clearly that changes were induced when ventriculin was given. The percentage distributions, according to cell diameters, for all corpuscle measurements in the blood of control animals as well as those obtained from all test groups are shown in table 2 and the Price-Jones curves of the percentage distribution of red cells in the control and of the percentage distribution found in the blood of young born to mothers fed the diet for 14 days are shown in figure 1.

It will be seen from table 2 that the highest frequency in the percentage distribution of the diameters of the red cells of control animals occurred in the column designated $10\ \mu$ and that high percentages likewise occurred in the columns designated 9.0 and 9.5 respectively. When the diameters of the

red cells in dry smears of blood of the test animals were tabulated for all groups there was a gradual shift in the higher percentages to the left, showing thereby higher percentages of cells of smaller size in the blood stream of newborn rats which were born to adults eating the diet containing the antianemic principle ventriculin.

TABLE 1

Comparison of number and size of red cells at birth in injected and control groups

DAYS OF GESTATION PERIOD ON RATION CONTAINING VENTRICULIN	NUMBER OF NEWBORN RATS	CUBIC CENTIMETERS OF PACKED RED CELLS PER 100 CC. OF BLOOD	ERYTHROCYTES	
			Number per cubic millimeter, millions	Largest diameter, μ
Controls	177	44.8 $\pm$ 0.3 (32.0 — 55.0)	3.04 $\pm$ 0.02 (2.26 — 3.72)	9.42 $\pm$ 0.03 (8.83 — 10.32)
Last 7 days	18	40.5 $\pm$ 0.7 (31.0 — 49.0)	2.90 $\pm$ 0.04 (2.45 — 3.50)	9.40 $\pm$ 0.06 (9.02 — 9.86)
Last 10 days	19	39.8 $\pm$ 1.2 (25.0 — 52.0)	2.76 $\pm$ 0.07 (2.13 — 3.49)	9.07 $\pm$ 0.02 (8.76 — 9.45)
Last 14 days	31	38.9 $\pm$ 0.6 (31.0 — 46.0)	3.36 $\pm$ 0.04 (2.80 — 3.91)	8.80 $\pm$ 0.02 (8.08 — 9.95)
Entire term	57	43.7 $\pm$ 0.4 (35.0 — 50.0)	3.09 $\pm$ 0.04 (2.24 — 3.98)	8.72 $\pm$ 0.05 (8.04 — 9.55)

It seems clear from the curves of the percentage distributions of diameters of all cells measured for the control animals and for the offspring of mothers eating the ventriculin diet for the last 14 days of term (fig. 1) that there is a marked shift of the higher percentages to cells of smaller diameters. The antianemic substance, ventriculin when fed to pregnant rats has apparently had some effect on accelerating the maturation of the red blood cells in the developing fetuses.

COMMENT

Our data indicate that the antianemic preparation, ventriculin, when fed to pregnant rats during gestation, has some

TABLE 2
Distribution of the diameters of the erythrocytes of newborn rats whose mothers had been fed a ration containing ventriculin and of control rats whose mothers had been fed a ration not containing ventriculin

DAYS OF GESTATION PERIOD ON RATION CONTAINING VENTRICULIN	NUMBER OF LITTERS	NUMBER OF NEWBORN RATS	NUMBER OF CELLS	PERCENTAGE FREQUENCY													
				Microns													
				6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5
Controls	25	177	17,700		0.003	0.02	1.55	10.37	13.48	17.90	17.56	20.85	12.18	2.52	2.55	0.78	0.003
Last 7 days	2	18	1,800	0.11	0.11	0.44	1.22	9.44	14.55	18.44	22.88	19.11	8.00	3.00	1.00	1.44	0.22
Last 10 days	3	19	1,900		0.42	0.10	3.68	11.47	27.47	7.89	25.78	16.21	6.00		0.94		
Last 14 days	4	31	3,100			6.19	7.80	19.62	17.22	18.12	11.03	12.25	5.35	0.70	1.29	0.45	
Entire term	7	57	5,700	0.10	0.14	5.22	7.54	23.01	13.96	24.00	10.03	12.77	1.50	1.43	0.24		

effect on the hemopoietic system of the fetus, in that the diameters of the red corpuscles in the newborn are smaller than corresponding figures for controls animals. The results are similar to those observed when gastric juice from human or swine sources was injected intraperitoneally into pregnant rats. Wigodsky and Ivy ('38) studied the blood of newborn rats whose mothers had received injections of a potent liver extract. They did not observe any changes in the average diameters or volumes of the erythrocytes.

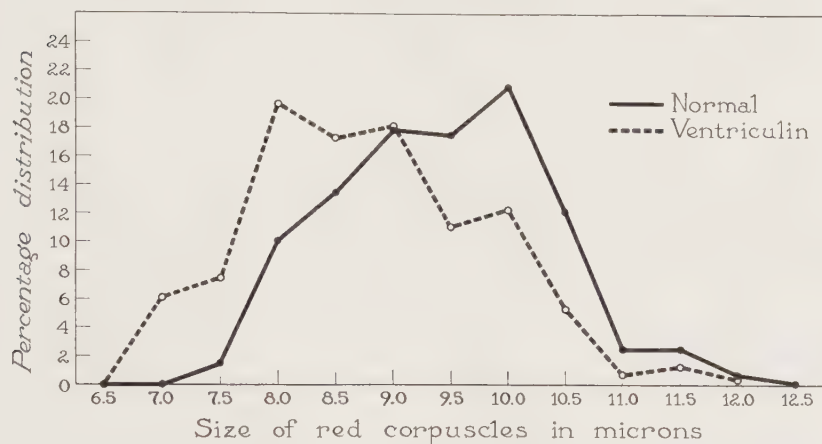


Fig.1 Percentage distribution of the erythrocytes, according to size, in newborn rats of control mothers and of those fed a diet containing ventriculin during the last 14 days of term.

The variability of our data, however, warrants consideration. In young rats born to apparently healthy adults which had not received ventriculin the total number of red cells per cubic millimeter of blood ranged from 2,260,000 to 3,720,000. The diameters of these cells measured in dry smears, ranged from $8.83\ \mu$ to $10.32\ \mu$. Thus the variability of such data in normal animals is great and presents considerable difficulty in properly evaluating the results obtained from studying the red corpuscles of the test animals. If the supposition is correct that the maturation of the fetal red blood corpuscle depends on maternal sources of some hemopoietic

principle (whether the antianemic principle of Castle or not), then the wide ranges in our control data probably depend on variations occurring in these maternal stores.

Varying degrees of anemia occur in adult rats during pregnancy. This would seem to be due to variations in the reserve of hemopoietic regulatory principle available in the adult, together with the demands made on the maternal organism by the developing litter of young. There may be some definite correlation between the total number of erythrocytes in the newborn and the degree of anemia which develops in the mother during pregnancy. This relationship is being studied.

The smallest number of erythrocytes per cubic millimeter of blood (2,240,000) found in newborn rats born to mothers which ate ventriculin for the entire term was as low as occurred in some of the control animals. But the largest number (3,980,000) in this group was considerably higher than in any of the controls. Likewise when contrasted with that of the controls, decreases in the corpuscular sizes in the blood of test rats were apparent. The difference between the mean of 17,700 measurements of cells in control animals and those made on the corpuscles of test animals whose mothers had been maintained on the ventriculin diet for the entire term of pregnancy was $0.70 \pm 0.06 \mu$, or over eleven times its own error. This would appear to be a significant reduction in the size of the red cell.

SUMMARY AND CONCLUSIONS

The antianemic substance, ventriculin, was mixed with a normal standard rat ration and fed to pregnant white rats during a part or all of their gestation period. Blood was taken from the heart of their offspring at birth or very soon thereafter and a study was made of the diameters of the red corpuscles. Cells were measured directly from paper negatives of dry smears. One hundred cells were measured from each smear and the average was determined. Data obtained from 177 newborn rats born to mothers which were fed the standard ration served for control. Observations

were made on the blood of 125 newborn test rats which were born to mothers fed ventriculin. The following conclusions were drawn:

1. Ventriculin, a stomach preparation, contains some substance which when present in the maternal organism usually accelerates the maturation of the blood cells in the developing fetus. It seems likely that this substance passes the placental barrier.

2. A wide range in the effectiveness of the preparation was encountered. Some test litters of young were observed to have red corpuscles as large as those of the controls. It appears that some animals are more refractory than others to the action of this antianemic substance.

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THE COMPARATIVE ANATOMY OF THE DORSAL INTEROSSEOUS MUSCLES

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FIVE FIGURES

Through curious coincidence, Ruge and Cunningham in 1878 independently published widely divergent theories of the homology of the human dorsal interosseous muscles. These theories have never been evaluated on grounds common to both and now, 60 years later, two opposing schools trace their viewpoints to one or the other of these pioneers. The investigation upon which this paper is based stands as the first attempt to reconcile the two systems. It turned out that while Ruge's theory is consistent with all previously known facts and with those unearthed in this endeavor, the view of Cunningham is invalid because of a major error in his interpretation of the anatomy of the marsupial hand. But an introductory historical resume is necessary first to define the problem.

Cunningham (1878 a) dealt with the hand of marsupials. The substance of his report was that the short intrinsic muscles of the hand are divided into three layers: the first being the palmar group in which he erroneously included the palmar interossei with the adductors (contrahentes); the second layer being the flexor brevis sheet, embracing in the human hand only the flexor pollicis brevis and the flexor digiti quinti brevis; and the third or dorsal layer being made up of the marginal abductors as well as of the dorsal interossei (fig. 5, A). In a second paper (1878 b) he considered the foot of

placental mammals as well as that of marsupials and concluded that "the typical arrangement of the muscles of the pes is the same as in the hand, and that this arrangement is to be seen to best advantage in the feet of certain marsupials." Young (1880) extended Cunningham's work and strongly upheld his conclusions. The theory was again examined by McMurrich ('03, '07) who added certain modifications. In a rather sparse phylogenetic series including amphibians, reptiles, and mammals he found an apparent evolutionary continuation of certain intermetacarpal muscles found in the extremities of lower forms with the dorsal interossei of man and other mammals. But to bring the theory into agreement with the indisputable evidence of Ruge and the embryologists whose work will shortly be discussed, he stated that the dorsal interossei are composite structures formed from the dorsally placed intermetacarpal anlagen as well as from portions of certain flexores breves profundi which migrate up from below in the course of development. Ribbing ('09) in an extensive treatment of the phylogeny of the muscles of the shank and foot reaffirmed McMurrich's views. Since then this thesis has been supported by many workers including Stjernman ('32) and Howell ('36).

Ruge (1878 a) approached the problem in an entirely different way than did Cunningham. He studied the embryological development of the dorsal interosseous muscles in the foot of man and claimed that they arise ventrally from anlagen which lie in the same horizontal plane as do those of the ventral interossei and that they attain their dorsal position by migration. This migration he observed, with the exception of that of the first dorsal interosseous muscle. He described no dorsal anlagen or intermetatarsals (fig. 5, B). In a second paper (1875 b) he pointed out that this migration may be seen phylogenetically in the primate group and also quotes Champneys to that effect. In a little known paper, Windle (1883) investigated the embryology of the short muscles of the human hand. He found the dorsal interossei to be derived from ventral precursors and to achieve their adult

position by migration—thus strongly supporting Ruge's views. In 1900, Schomburg published the results of an ontogenetic study of the human foot and besides confirming Ruge's conclusions added that he found the first dorsal interosseous muscle to arise ventrally as do the others. Gräfenberg ('05) in a very detailed study of the development of the human hand again called attention to the fact that the dorsal interossei arise in continuity with the ventrals. He observed a plate of muscle primordium which contained the anlagen of all the interosseous muscles, dorsal as well as ventral. This, according to his description, splits up to form the forerunners of the adult structures. Further substantiation was added by Bardeen ('07) who repeated the work of Ruge, Windle, and Schomburg in his study of the development of the lower extremity in man.

It is plain that these two opposing concepts are based upon different sorts of evidence. That there are basically two layers represented in the interosseous muscles is an idea which is to be traced back to Cunningham's interpretation of the anatomy of the marsupial hand. Embryologists, on the contrary, allow only a unilaminar derivation of these muscles. It seems obvious that the proper grounds upon which to attempt reconciliation of the two camps is an ontogenetic study of the marsupial hand. And in the following pages a report of such a study is given. This will involve, of necessity, a description of the muscles of the adult as well.

In the opossum hand, the three lateral interosseous spaces are occupied by muscles which have hitherto been described as dorsal interossei. These arise from the sides of adjacent metacarpals, thus filling the entire hiatus between the bones (fig. 1, flex. dig. minimi). They are powerful muscles and each ends in a short stout bifurcate tendon which inserts through the joint capsules and the sesamoid bones on the basal phalanges of both neighboring digits. Their innervation is by means of the deep ulnar nerve, proving that they are derivatives of the ventral muscle mass. In the small pouch young these muscles are seen to consist of two components, each of which arises on the metacarpal and inserts

on the basal phalange of its respective digit. Thus one muscle in each interosseous space is an abductor, the other an adductor.

In reality, it is seen, there are six dorsal muscles in the newborn opossum hand, for curiously no traces of these muscles are to be found in the first interosseous space. That these structures should not be called dorsal interossei follows not only from the fact that adductor as well as abductor elements are present but also because they have entirely different relationships to the deep short flexors. Even in the very early stage (14 mm.) there is no evidence of migration from below.

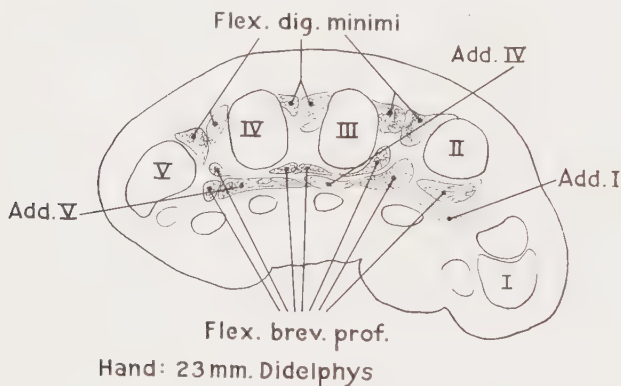


Fig.1 Cross section of hand of 23-mm. opossum pouch young.

And in addition there is, to correspond to each of these muscles, a well-developed flexor brevis profundus. These are seen in the adult as small muscles arising from the deep carpal ligaments and the volar surfaces of the metacarpals and inserting on the sesamoid bones at the metacarpophalangeal joints. In the ontogenetic series they arise in situ and in the 23-mm. pouch young they exhibit relations similar to those in the adult (fig. 1).

In the grown opossum there is a muscle arising from the base of the first metacarpal, from the deep carpal ligaments, and from the entire radial surface of the second metacarpal. This inserts on the basal phalange of the second digit. In all respects it resembles the first dorsal interosseous muscle of

the human hand and study of the ontogenetic series shows that like that muscle it develops entirely from the radial head of the deep short flexor of the index finger. In the 14-mm. pouch young this structure is found lying on the volar surface of the second metacarpal, but as larger and larger specimens are examined, its extension up into the first interosseous space is clearly seen. In cross sections of the large pouch young it may be distinguished from the dorsal muscles occupying the lateral three spaces in that it does not extend quite as far dorsally, and unlike the lateral muscles it covers part

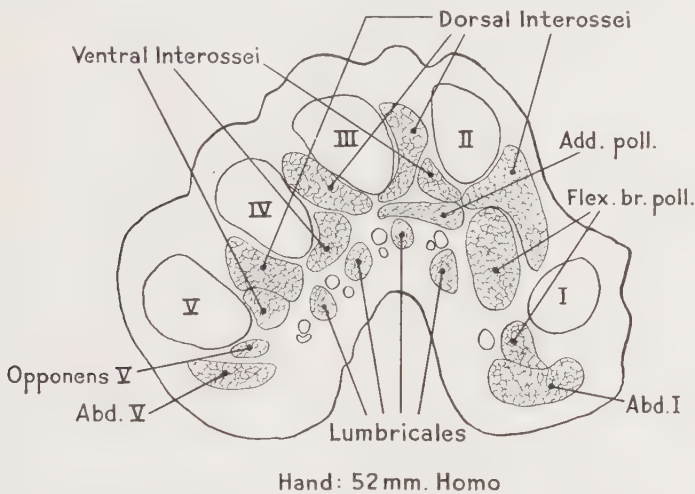
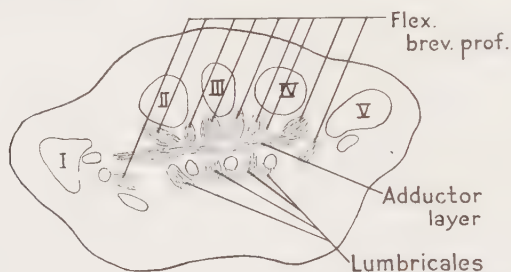


Fig. 2 Cross section of hand of 52-mm. human embryo.

of the volar surface of the metacarpal. Its similarity both in the adult state and in its development makes this muscle the undoubted homologue of the first dorsal interosseous muscle of the human hand. It will be seen, however, that while in respect to the first interosseous space the muscles of the opossum and man are similar, in the lateral three spaces there are no structures to correspond to the human dorsal interossei number two, three, and four (compare figs. 1 and 2).

It is not possible to homologize the dorsal muscles of the lateral three interosseous spaces of the opossum with structures described in lower forms. McMurrich's identification

of the dorsal elements of the 'interosseous' muscles of the opossum—undoubtedly including the muscles under consideration—with the intermetatarsal muscles of *Reptilia* lacks proof. Besides, it stumbles on the fact that the presence of both abductor and adductor muscles in the same space is a peculiarity of the marsupial. In hopes of shedding some light on this complicated problem I have begun a study of the development of the short muscles of the turtle hand and foot. Tentatively it seems reasonable to call these muscles flexores digitorum minimi—a term introduced by Ribbing to denote certain short flexors of the first phalanges in *Amphibia*.



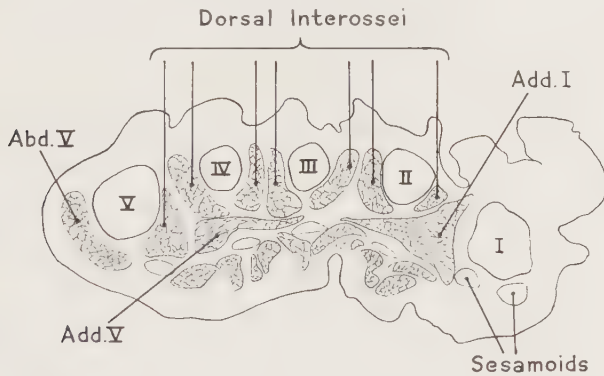
Foot: 23 mm. *Didelphys*

Fig. 3 Cross section of foot of 23-mm. opossum pouch young.

A cross section of the foot of a 23-mm. pouch young (fig. 3) shows that while a full set of short deep flexors is present, no trace of the flexores digitorum minimi can be detected. In progressing from this early stage to those more adult, the development of abducting dorsal interosseous muscles by differential migration or extension of these anlagen may be observed in all four interosseous spaces. This occurs as described by Ruge and Schomburg. In the adult opossum, consequently, the four dorsal muscles are entirely homologous with those of the human foot.

Examination of sections of the hands and feet of diverse mammalian species indicates some interesting trends in the development of the dorsal interosseous muscles. In the series

available for such study only the shrews, the tapir, and the artiodactyls retain the primitive ventral position of all the deep short flexors. At the opposite extreme is the foot of *Myotis*, a bat, in which all of the short deep flexors (with the exception of those marginally placed) have extended upward to become dorsal interossei (fig. 4). That this is not true in all chiroptera is evident from the figures and text of the excellent paper of Anthony and Vallois ('14). From *Myotis* we may draw the conclusion that a potentiality for this upward extension is shared by all of the deep short flexors. And this conclusion is nicely illustrated in the feet of primates.



Foot: Adult *Myotis*

Fig. 4 Cross section of foot of adult *Myotis*.

According to Straus ('30) the usual primate arrangement shows the dorsal interosseous muscles centering around the third digit (fig. 5, F). This means that the abducting muscles of the second and fourth digits have shifted upward along with both muscles of the third digit. In certain prosimians the muscle which comes to occupy the third interosseous space is not the fibular head for the third toe, but the tibial head for the fourth. Thus the center of abduction is shifted to the fourth toe (fig. 5, E). In some chimpanzees, the majority of gorillas, and in most men, on the contrary, the muscle of the second digit, rather than of the third, passes dorsally in the



Fig. 5 Diagrams of intrinsic muscles of various hands and feet. A. Cunningham's scheme for the mammalian hand. a, flexor pollicis brevis. b, flexores breves profundes. e, flexor digiti quinti brevis (a, b and e together form his second or middle layer). d, palmar interossei. e, dorsal interossei. B. Scheme of Ruge's interpretation of the human foot. C. Scheme for the opossum hand. f, flexores digitorum minimi; g, rudimentary flexor brevis profundus. D. Scheme for bat foot. E. Scheme for prosimian hand (according to Straus). F. Scheme for usual primate foot. G. Scheme for foot of Australian native.

second space and the axis is consequently shifted to the second toe (fig. 5, B). In my study of the foot of the Australian ('36) an intermediate condition was described in which both muscles corresponding to the second space had extended dorsally with a consequent disruption of the axis of the foot. The figure accompanying the description of the Australian foot should be compared with that of the bat foot, for in respect to the second interosseous space, the conditions are the same (fig. 5, D and G). Wells ('31) has shown that in the South African native variation in the pattern of the short deep flexors is not uncommon.

From the evidence detailed above we are obliged to conclude that the dorsal interosseous muscles, wherever they exist, are derived from a single layer of anlagen which also gives rise to the ventral series. The work of Cunningham, which unfortunately took as a starting point the hand of the marsupial must be discarded in favor of the theory of Ruge and of the embryologists who later substantiated his findings. It is interesting to speculate that as the foot of the marsupial closely follows the usual mammalian pattern, while the hand does not, that had the order in which Cunningham considered the extremities been reversed he would have arrived at different (and probable tenable conclusions). McMurrich's modification of the Cunningham theory is no more satisfactory than is the original. The intermetacarpal muscles which he postulated have no mammalian counterparts. Except in position, there is no similarity between the structures which I have called the flexores digitorum minimi and the rudiments he claimed are present generally in mammals. Instead of looking on the dorsal interossei as composite structures we should rather define these structures as follows: the term dorsal interosseous muscle denotes any extension of the flexores breves profundi into the interosseous spaces.

SUMMARY

1. A reinterpretation of the marsupial hand on the basis of embryological studies has given an entirely different idea of its anatomy (as regards the deep intrinsic muscles) than that offered by Cunningham. Furthermore the findings harmonize with the concept of Ruge and his followers, Windle, Schomburg and Gräfenberg.

2. Short flexor muscles, tentatively called flexores digitorum minimi, are described in the lateral three interosseous spaces of the opossum hand. These are not found in the first space and are entirely absent in the foot; nor are these structures to be found in the hands or feet of placental mammals.

3. The unusual condition of the dorsal interosseous muscles in the foot of the bat, *Myotis*, where instead of one such muscle to a space there are two, is described. From this it is concluded that each of the flexores breves profundi has the potentiality for extending dorsally into the interosseous spaces. This fact clarifies the shift in the axis of abduction which is encountered in the feet of primates and has been seen in the foot of the Australian and of the South African native.

4. The term dorsal interosseous muscle is defined as the dorsal extension into the interosseous spaces of any of the flexores breves profundi.

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BOOK REVIEW

A STEREOSCOPIC ATLAS OF THE RAT EMBRYO—13½ and 15 day stages. By J. A. Long, Ph.D. Published by the California Laboratory Supply Co., Berkeley, California. 53 stereoscopic plates. Price (without stereoscope), \$4.50.

A year ago, when reviewing the recently issued atlas of the chick embryo by Long and Burlingame, the writer expressed the hope that the projected atlas of a mammalian embryo would soon be forthcoming. This expectation has now been happily realized.

The present series of plates cover the detailed anatomy of the 8- and 10-mm. stages in the development of the rat, i.e., the critical period in organogeny. This is unfolded in a superb series of flawless photographs displaying, in surprising detail, successive levels of dissection. Much ingenuity and good judgement has been shown in supplementing such full length views with higher-powered views of regions taken from significant angles. Finally there are twelve plates displaying translucent embryos injected with India ink.

If one were to pick out special features in so uniformly excellent a series, mention might be made of the ventral dissections of the heart and bulbus cordis (particularly to show the development of the bulbar septum), the cerebral blood vessels in relation to parts of the brain, the sculptured membranous labyrinth, the developing choroid folds in the ventricles of the brain, the partitioning membranes of the coelom, the circulation through the lobate lung and the topographic relations of cerebral and spinal nerves.

In conclusion, it can be stated that this atlas is not merely an aid to laboratory study but that it adds another to that comparatively small number of studies of mammalian embryos in which the topographic anatomy has been fully disclosed—namely, the opossum, pig and man. With this delicate technique so thoroughly mastered, it is to be hoped that Professor Long will not stop until he has presented us with a stereoscopic atlas of the human embryo.

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MICROPHYSICAL CHANGES INDUCED IN STRIATED MUSCLE AFTER CAFFEINE IMMERSION

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TWO PLATES (FOURTEEN FIGURES)

The details of the sequence and nature of the structural changes associated with striated muscle contraction and contracture are still matters of controversy. Obviously, both physical and chemical factors are involved. The histophysiological effects of caffeine upon the microphysical changes have not been reported. Considerable explanation has been given concerning the chemical events and their sequence in the single muscle twitch and in normal tetanus. It is also well known that striated muscle shortens when excised from its natural attachments, a condition which may pass into a state of myostatic contracture (Davenport and Ranson, '30) if it is allowed to persist as in tenotomy. Prolonged shortening and delayed relaxation have been reported in striated frog muscle after treatment with veratrine, nicotine, and acetylcholine. Caffeine has been shown to affect the physiological behavior of striated muscle (Cheney, '31, '32 a, '32 b). It is the intent of this paper to describe the histological changes occurring in the myofibrillae and in the muscle fibers in toto in response to the immersion of striated muscle in caffeine solutions of different concentration which produce graded effects from ineffectiveness to contracture.

Recently (Speidel, '38) and also (Speidel, in press)¹ in an exhaustive study of the histo-physiology of the process of

¹ Under the title, "Some features of contraction nodes and retraction clots, as observed in single fibers of cardiac and skeletal muscle of both vertebrates and invertebrates," Speidel has presented definitions of these terminologies in the October, 1938 issue of the *Biological Bulletin*.

growth, injury and repair of living striated muscle in the frog tadpole, has introduced terminologies which apply accurately to the microstructural states which I have observed with caffeine effects. He distinguishes three varieties of clots depending upon the degree of disorganization and injury of the myofibrillae involved. These terms will be used in this paper. I will employ also certain other phrases which are in accord with the terminologies referred to above. I mean such references as the 'incipient contraction clot,' the 'partial or local contraction clot,' and the 'contraction or retraction clot or cap.' The 'wavy' appearance of the fiber is the result of the folding of the myofibrillae due to a loss of tension. This is a variation within the normal and is not the result of caffeine action. However, any bend in a myofibril does favor clot formation and incipient clots develop very commonly after caffeine at such areas. The retraction clot involves the breaking of the myofibrillae in considerable numbers and thereby severing the continuity of the myofibrillae of the fiber. Such severe clots are probably irreversible thereby sealing the entire fiber transversely and preventing further retraction. All degrees of the loss of alinement of the Q and J band material up to the true retraction clot are graded stages of the so-called incipient clot.

The extreme retraction clot stage is the condition referred to originally by Louis (1830) and described in detail by Zenker (1864) as a common type of muscular lesion coincident with fatal typhoid fever in 120 human cases. Zenker found these striated muscle lesions abundant in the rectus abdominis and in various adductor muscles. Currently, this condition is called Zenker's waxy degeneration and refers to a structural state often seen in striated muscle as a result of several other pathological phenomena such as tetanus toxin effects, pneumonia, and influenza. This development of muscular lesion occurs also after the injection of powerful irritants such as phenol or boiling water (Forbus, '26).

A series of papers (Nageotte, '24; '25 a, b, c; '29; '37) dealing with the microphysical changes in striated muscle contractions, as demonstrated by fixed and stained preparations

after treatment by such environmental factors as faradic current, heat, cold, and chloroform, are also especially illuminating and of interest due to the amazing similarity of several of the caffeine effects presented here.

The striated muscle used throughout my experiments was the gastrocnemius of *Rana pipiens* Schreber. It should be recalled that the detachment of the muscle results in a degree of shortening which is a response to handling, to the release of tension and probably also to the fact that even carefully prepared Ringer is not absolutely a normal environment. As a consequence, some of the fibers in every so-called normal preparation are somewhat affected. These effects are reduced to a minimum by allowing the muscle to rest in Ringer for a 5-minute period before it is subjected to any experimental condition. This histological picture was taken as normal for comparison with caffeine effects with reference to the relative number of fibers affected. The wavy contours of various fibrils are common. The excised gastrocnemius was measured in the Ringer and transferred to the caffeine-in-bicarbonate-buffered Ringer solution for time periods varying from 2 seconds to $\frac{1}{2}$ hour. Then the muscle was measured in millimeters to ascertain the amount of shortening which resulted from the immersion. Since fixation in the exact state of the muscle coexistent with its precise immersed condition was impossible because a definite, but unavoidable and slight handling or transfer effect; and, also in recognition of the fact that Nageotte ('37) noted some recovery momentarily in the slightly affected myofibrillae and considerable change even when fixed within 1 minute after the cessation of stimulation, I attempted to standardize conditions for comparative effects. It was noted that the muscle length existing when immersed in caffeine would recover in Ringer within 2 minutes usually. It always recovered within 5 minutes unless the effect upon the fibers was drastic as shown by the sections. If the recovery length from caffeine-Ringer required more than 5 minutes to regain the recorded length in the original Ringer, it would not recover its length at all or

at least not within $\frac{1}{2}$ hour. Therefore, the muscle was rested in Ringer for 5 minutes, then subjected to the caffeine solution for a given time, then washed for 5 minutes in Ringer, then pinned to a block without altering the existing length, and fixed in Bouin's. Heidenhain's iron haematoxylin was the stain employed as being most satisfactory for the details of the myofibrillae and for reproduction by photomicrography.

The foregoing procedure may miss some immediate and very temporary effects which can be studied only in the living fiber, but it standardizes the histological picture so that any effects persisting more than momentarily and due to the caffeine action upon the sarcoplasm or myofibrillae can be studied in the muscle. The subjection of the muscle to various concentrations produced changes ranging from no effect to those of full contracture. The nature and relative extent of the physical changes induced by the action of the same concentration for varying periods can be compared.

Figures 1-a to f inclusive presents photomicrographs of the normal and variations within the normal which occur in the fibers by mechanical handling of the mass muscle, the loss of tension accompanying excision of the muscle from its natural attachments, and the unavoidable absolute non-isotonicity of the Ringer.

The immersion muscle series consisted of subjecting the muscle to 0.03% caffeine (alkaloid)-in-Ringer for 10 minutes and for $\frac{1}{2}$ hour. The second series involved 0.06% RC and the time was $\frac{1}{2}$ hour. The third series was 0.12% RC for 10 seconds, and the fourth series was in 0.5% RC for 2 seconds. The fifth series was in 1.0% RC for $\frac{1}{2}$ hour and produced contracture. Photomicrographs of the effects of these direct contacts of the muscle with caffeine are shown in figures 2-a to h inclusive.

The shortening of the entire muscle does not coincide with the extent of the myofibrillar changes because the structure of the sarcolemma and the muscle fascia obviously interfere. Moreover, the shortening of the myofibrillae cannot exceed the extent permitted by their molecular structure which is different from that of internal perimysium and of the fascia.

The 0.03% caffeine produced no visible wrinkling of the fascia although folded myofibrillae (wavy areas, non-caffeine effect) and a number of partial or local incipient contraction clots are observable in some fibers (fig. 2-a). It has been found (Frank, '27) that the ratio of the width of the isotropic J bands and anisotropic Q bands was the same in resting muscle, in complete fatigue, rigor mortis, and in chloroform contracture but this ratio was not the same as the ratio *in* varying degrees of contraction. Later, it was shown (Hürthle, '30) that different fixatives augmented the contraction caused by the electrical stimulation so he did not believe the ratios stated by Frank gave a true picture of the living muscle. Nageotte ('25 a, c) found that chloroform vapors or a faradic current caused a mottled appearance of the fibers which he interpreted as the contraction of myofibrillae in spots since it could be prevented if the fibers were stretched slightly before fixation. Repeating Nageotte's work, Gavrilescu ('29 a, b) confirmed the report for fixed material but in fresh muscle stimulated to contracture and examined in physiologic saline, he saw no alteration in striation. The fibers appeared to be normal.

However, this mottling and blurring effect regarding the cross striations has been noted (Davenport and Ranson, '30) and also the more pronounced longitudinal fibrillation was observed in the rat's gastrocnemii in contracture produced by tetanus toxin (Davenport, Ranson and Stevens, '29). The blurring effect can be seen readily after caffeine action (fig. 2-e) and also in irritated areas of the so-called normal muscle mass (fig. 1-f). Nageotte ('37) shows similar effects after extreme faradic current stimulation. I have seen it commonly after caffeine immersion.

This blurring of the cross striations is due to a loss in the physical alinement of the myofibrillar Q and J bands; and probably also in part, to the chemical changes occurring in the sarcoplasm of the fibers. The caffeine, or any other source of stimulation, reaches the sarcoplasm and inaugurates processes resulting in the redistribution of myofibrillar substances. Such chemical differences would cause variations in

the staining properties and differences in staining *do* occur in adjacent areas. An examination of the slightly blurred areas (fig. 2-e) under high magnification shows that the same Q and J bands as a whole can be traced in some instances across dark, light, and non-staining areas. No predictable relationship exists between the intensity of staining properties and the normal or abnormal width of the Q and J bands. Mottling in similar areas occurs with various stains so it is not a case of poor differentiation with iron haematoxylin. In normal or slightly stretched myofibrillae (fig. 1-b, c) the Z membranes can be seen and the Q and J bands of adjacent fibrils are in alinement. At well-focused areas, they can be seen to be continuous. With a loss of alinement after fairly concentrated caffeine action, similar to any drastic and prolonged stimulation, there is apparently a rearrangement of the substance of the Q and J bands and a rupture of the Z membrane interfibrillar continuity. This situation results in strongly blurred striations, mottled staining, incipient contraction clots and the early or weak retraction clot stage—the degree of effect varying with the sensitivity of the individual or group of myofibrillae, to the causative agent of stimulation.

The wavy contour of some fibrils (fig. 2-a, f, g) is the fixation picture of the condition characterized as folded myofibrillae without loss of alinement of Q and J materials. At rest and in the contraction band state, without any caffeine action, the isotropic and anisotropic bands are in transverse parallelism; but, whenever there is a loss of tension in one or many fibrillae, a consequent shortening of some fibrils occurs. This condition causes an unequal stress with adjacent myofibrillae and a folding process which is seen as a wavy effect.

If a definite caffeine effect is produced, minute areas of fibrils stain differently (a little deeper and larger than normal) and appear as dots, due to a slight chemical and physical alteration of a localized area of the Q and J band substance in a single fibril; or, a mass of similar changes in

approximating fibrils and therefore appearing as a more extensive but slight differentiation. Both conditions are incipient clots. Increased chemo-physical disruption and local loss of alinement by further shifts in the original integrity of the myofibrillar substance produces the histological picture of the darkly stained linear areas extending incompletely across the fiber—see portions of figures 2-a, c, f, g. Such areas are indicated by the terminology ‘incomplete or partial contraction clots.’ A few myofibrillae may be broken or torn apart. Later and more drastic stages of clotting or darkly stained areas forming completely across the fiber form the true ‘retraction clot or cap’ (fig. 2-h) which are always abundant in contracture. Many fibrils are actually ruptured. The graded congealing effect is referred to by Nageotte ('37) under the word ‘congelation’ which impresses me as a very excellent term.

It is of interest that the histological appearance of the physical changes caused by the higher concentrations of caffeine are apparently identical with similar extreme contraction and contracture states shown by Nageotte ('37, p. 606, fig. 3-a, b; p. 610, figs. 4, 5 after extreme faradic current contraction; and, p. 621 figs. 15, 16 after chloroform contraction). It is the pre-dissolution stage of Zenker's waxy degeneration phenomenon. Corresponding effects caused by contracture after tenotomy are seen in the photomicrographs of Davenport and Ranson ('30, p. 12). When the congelation extends to a degree in which the continuity of the myofibrillae is completely broken and the terminal or completely transverse areas of the fiber are sealed, the term ‘retraction clot or cap’ has been applied by Speidel ('38) and shown to occur in fibers in the living animal (Speidel, '38, p. 189, fig. 5; p. 191, fig. 6). The higher concentrations of caffeine produce such areas (fig. 2, h) commonly in the caffeine contracture of individual fibers. Of interest here is the fact that such retraction caps (clots) will occur not only after caffeine immersion but after almost any type of injury (Speidel, '38) such as cutting, bruising, salt solutions, sugar solutions,

thyroid extract, heat, starvation, alcohol, and many other chemical reagents. They are seen also in normal metamorphosis in tadpole material (Speidel, '38).

It has been reported (Nageotte, '37) that all stages of this clotting (congelation) phenomena are reversible when due to faradic current stimulation but irreversible when produced by a volatile anaesthetic such as chloroform. The histological pictures, subsequent to extreme contraction and clot formation, are very similar and no adequate explanation is given for the difference of reversibility and irreversibility under the two conditions. It is quite possible that there is a sufficient diversification in the chemical events preceding the clot formation to account for the difference in reversibility. However, it has been shown (Speidel, '38) in the living fiber, that *all* retraction caps are followed by a dissolution stage at that zone of all parts within the sarcolemma and complete regeneration of the fiber, rather than reversible recovery directly. This regeneration phenomenon requires several days to regain normalcy (cross striation reappearance). Non-clotted areas of a fiber with clots in certain zones may remain normal and do not necessarily undergo dissolution.

After immersion in caffeine, prompt recovery occurs within seconds or minutes with respect to its ability to respond to another stimulation upon return to Ringer when the muscle is subjected to a brief exposure to lower concentrations such as 0.03% (fig. 2-a). If the caffeine effect, however, reaches even the incipient clot stage, it is probably many hours before the cross striations are regained. When immersed for $\frac{1}{2}$ -hour period (fig. 2-b, d) or in higher concentrations for an interval sufficient to cause contracture (fig. 2-h), full recovery did not occur nor even relaxation of the fibers in cases of contracture, at least not within a period of several hours in Ringer. I am inclined to agree therefore with the interpretation of probable irreversibility (Speidel, '38) with reference to severe contraction or retraction clots or caps and that a time interval must be allowed for the regeneration of the sarcomeres involved, before normalcy is re-established. This belief is substantiated by the kymographic record obtainable after

injury or by treatment by injection of medium concentrations of caffeine as well as by the histological examination of such muscle. The effect of caffeine by injection upon muscle behavior physiologically and the corresponding histological changes will be discussed in a subsequent paper. Probably the lighter-stained areas of incipient local clots (congelations) are rather rapidly reversible or regenerated quickly to the normal state although I have no direct evidence.

Immersion of striated muscle in the weaker concentrations of caffeine (fig. 2-a, b, c, d) for a considerable interval (10 to 30 minutes) or in a stronger concentration (fig. 2-f, g) for a brief period (2 to 10 seconds) produce similar degrees of contraction clots.

SUMMARY

1. Various stages of contraction and ultimate contracture are induced in striated muscle (frog) by immersion in caffeine.

2. Muscle fibers showing a loss of alinement of the 'Q' and 'J' band material of the myofibrillae present different histological appearances in accordance with the degree of the loss of alinement which varies with the concentration of caffeine employed and with the time of immersion.

3. Caffeine concentrations of less than 0.06% produce primarily—unless the immersion is prolonged—the degree of contraction and loss of alinement of the isotropic and anisotropic bands indicated by the term 'incipient clot' of varying degrees.

4. Immersion for several minutes up to $\frac{1}{2}$ -hour periods in higher concentrations (ex. 1.0%) produced contracture which histologically shows an abundance of extensive ruptured myofibrillae forming retraction clots through the fiber in toto and drastic retraction caps at the tendon terminations of the muscle fibers.

5. Muscles subjected to lower concentrations (0.03%) recover their original length in Ringer—unless immersion is prolonged for $\frac{1}{2}$ hour. They do not recover after immersion in 1.0% after even a brief immersion. Histologically, there is a correlation between the presence of abundant retraction

clots and the action of higher concentrations and the absence of such clots in the muscles subjected to lower percentages.

6. The action of caffeine causes a more pronounced longitudinal fibrillation and local incipient clot formation, blurred cross striations, and the dissociation of Z membranes at interfibrillar areas coincidence with more complete stages of 'congelation' depending upon the concentration and immersion period.

7. Even with the most severe retraction caps formed during immersion in caffeine concentrations inducing complete contracture, the sarcolemma does not undergo disorganization.

8. The physical form of contraction and contracture due to caffeine, as indicated by the histological picture of the microphysical changes, is essentially no different from similar transformations of the microstructures of the myofibrillar 'Q' and 'J' materials and the Z membrane continuity in response to other types of stimulation. (To wit, this is true for cold, faradic current, and volatile anaesthetics (alcohol, chloroform) as reported by Nageotte ('37); also by the sequence of changes in the development of contracture resulting from tenotomy (Davenport and Ranson, '30); or by cutting, bruising, salt or sugar solutions, heat, thyroid extract, alcohol and starvation (Speidel, '38.))

9. It is suggestive that there is nothing specifically characteristic of caffeine action insofar as the nature of the microphysical changes are concerned. These microstructural alterations induced in striated muscle after caffeine immersion can be seen to involve the myofibrillae but they are in no way peculiar to caffeine stimulation. Apparently, although it is obvious that the many different substances or types of mechanical, thermal, chemical, and electrical stimulation undoubtedly produce individual chemical effects upon the sarcoplasm, the sequence and nature of the microphysical changes occasioned in the colloidal systems of the myofibrillae responsible for the actual shortening process, are identical (or very similar) with the changes inaugurated by widely different causative agents as revealed by the photomicrographs.

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PLATE 1

EXPLANATION OF FIGURES

1-a $\times 900$. Longitudinal, completely relaxed fiber showing Q and J bands, the latter narrow and Z lines (Krause's membrane) not evident. Common normal appearance.

1-b $\times 900$. Longitudinal portion of normal fiber showing Z lines in J bands of myofibrillae.

1-c $\times 2250$. Longitudinal, very slightly stretched myofibril showing Z membrane in J bands and Hensen's line in Q bands. Hensen's medium membrane is not obvious.

1-d $\times 440$. Transverse, portion of a fasciculus showing internal perimysium at 'a,' normal fibers at 'b,' Cohnheim area at 'c.'

1-e $\times 176$. Longitudinal, external perimysium or fascia at 'a,' wavy at 'b' and 'c' without rupture of myofibrillae, due to bending of myofibrillae. Area 'd' is quite normal.

1-f $\times 176$. Longitudinal, showing all conditions usually seen commonly (except area 'e') in fixed preparations of so-called normal excised muscle. Area 'a' is normal, 'b' slightly stretched, 'c' areas are incipient clots, 'd' wavy, 'e' retraction cap involving the rupture of the majority of the myofibrillae.

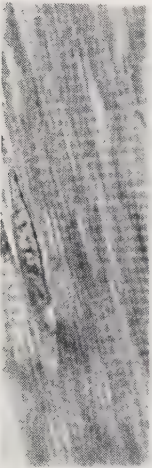


Fig. 1-a
900x

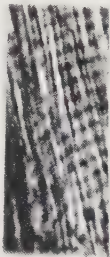


Fig. 1-b
900x

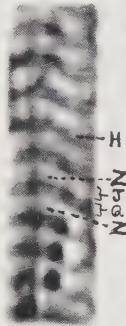


Fig. 1-c
2250x



Fig. 1-d
440x



Fig. 1-e
176x

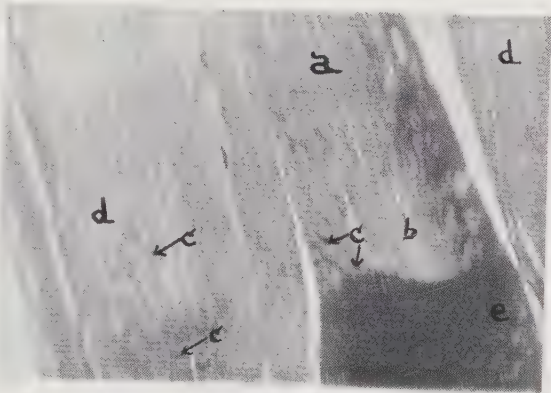


Fig. 1-f
176x

PLATE 2

EXPLANATION OF FIGURES

2-a $\times 176$. Longitudinal, after immersion in 0.03% caffeine for 10 minutes, portion of three muscle fibers, normal at 'a,' local incipient clots at 'b,' and wavy at 'c.'

2-b $\times 40$. Longitudinal, $\frac{1}{2}$ hour in 0.03% caffeine, showing internal perimysium at 'a,' abundant incipient contraction clots and early retraction clots at 'b' (note lack of uniform diameter of these clots across the muscle fiber), complete uniform retraction clot at 'c,' and definite retraction cap at 'd.'

2-c $\times 440$. Transverse section of figure 2-a (0.03% for 10 minutes) with normal fiber at 'a' and a fiber with local incipient clots at 'b.'

2-d $\times 100$. Transverse section of figure 2-b (0.03% for 30 minutes) with normal fiber at 'a,' and numerous clot stages, ex. 'e' areas.

2-e $\times 900$. Longitudinal, after 0.06% caffeine for 30 minutes, blurred areas at 'a' and 'b,' the result of some loss or breaking of physical alinement of cross striations and subsequent 'congelation' and contraction clot formation of various degrees depending upon the extent of physical disruption of normal structural relationships.

2-f $\times 100$. Longitudinal, after immersion in 0.12% caffeine for 10 seconds, showing normal fiber area at 'a,' wavy at 'b,' local incipient clot at 'c,' and the more extensive retraction cap stage at 'd.'

2-g $\times 100$. Longitudinal, after immersion in 0.50% caffeine for 2 seconds. Effect similar to figure 2-f but a tendency toward a greater degree of clotting with this higher concentration even with the very brief immersion. Normal region at 'a,' wavy at 'b,' clots at 'c,' retraction cap at 'd.'

2-h $\times 100$. Longitudinal, after immersion in 1% caffeine for 30 minutes, showing extreme development of contraction clots at 'a;,' retraction caps at 'b' and 'c;,' and at 'd,' a total disruption of the normality of cross striations structural continuity and full congelation of myofibrillar material with the sarcoplasm. This is the so-called final stage prior to dissolution of the waxy degeneration of Zenker and the nomenclature so common in pathological literature.



Fig. 2-a 176x

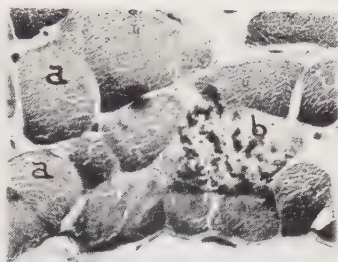


Fig. 2-c 440x



Fig. 2-b 40x



Fig. 2-d 100x



Fig. 2-e 900x



Fig. 2-f 100x



Fig. 2-h 100x



Fig. 2-g 1000x

HOMOTRANSPLANTATION OF SUPRARENAL GLANDS FROM PREPUBERAL RATS INTO THE EYES OF ADULT HOSTS ¹

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SEVEN FIGURES

Until recently, attempts to transplant the suprarenal gland met with little success and some (e.g., Oldberg, '29) were led to believe that it was impossible to obtain functional persistence of such transplanted glands. Since the work of Pencharz, Olmsted and Giragossintz ('31) the results have been more successful. It is now generally agreed that functional suprarenal grafts usually consist only of cortical tissue, the medullary part of the gland seldom persisting. It is well known that the interior of the transplants degenerates rapidly after the blood supply is interrupted and that the columns of cortical cells are restored by proliferation from a peripheral layer of zona glomerulosa or by the differentiation of less specialized cells in the capsule. Successful incorporation of the cortex does not occur in the absence of the capsule. A limited amount of evidence has been accumulated by different workers indicating that the extent of cortical replacement in suprarenal grafts is conditioned in some manner by the amount of cortical tissue present in the organism.

¹ These experiments were begun in the Department of Zoology at the University of Missouri during the summer of 1936 and completed at the Biological Laboratory, Cold Spring Harbor, Long Island, New York during the summer of 1938. It is a pleasure to acknowledge the provision of facilities by both of these institutions.

The present experiments were begun in order to extend the analysis of factors which regulate the incorporation and persistence of suprarenal transplants. A relatively large number of glands were transplanted into the eyes of diverse categories of hosts in order to evaluate statistically survival of the grafts subsequent to differentiation in animals possessing variable endocrine conditions. It was thought that the anterior chamber of the eye should be a more advantageous site than those which have been previously employed for suprarenal transplantation. The general characteristics of the grafts may be observed directly through the corneas. Moreover, the aqueous humor contains approximately the same metabolites as the vascular fluid and necrosis in the transplants should not be so extensive as in other sites since some of the nutritional requirements of the introduced tissues are provided previous to the establishment of blood vessels (Turner, '38). In addition to such observations as could be made through the corneas, histological and cytological techniques have been employed in order to study the cytomorphosis of the cortical elements during their differentiation and inward migration from the capsular region.

MATERIAL AND METHODS

In the following series of experiments, a total of 387 suprarenal glands were transplanted bilaterally or unilaterally into the anterior chambers of 245 hosts. All of the recipient animals were adults ranging from 60 to 186 days of age. The age of the donors varied from newborn to 32 days. Frequent observations of the transplants were made by means of a hand-lens or a dissecting microscope. The grafts were removed at intervals ranging from 12 hours to 5 months subsequent to insertion, the majority of them being allowed to persist for 3 months.

For histological observations, the grafts were fixed in Bouin's picro-formol, sectioned at 6 μ , and stained with Ehrlich's hematoxylin and eosin or with Mallory's triple stain.

The cytological material was fixed in the fluids of Champy and Helly, sectioned at 3μ , and stained with anilin-fuchsin-thionin-aurantia according to the method of Kull.

OBSERVATIONS

I. Homotransplantation of complete glands

Normal hosts. Since suprarenal transplants in intramuscular and intraperitoneal positions have been reported not to survive in normal animals (Wyman and tum Suden, '32; Ingle and Higgins, '38), a relatively large number of bilateral and unilateral grafts were studied subsequent to persistence in the anterior chambers of normal animals of both sexes (table 1 a). Most of these grafts were allowed to persist for 3 months and some for as long as 5 months. Ninety-six glands were transplanted bilaterally and thirty-nine unilaterally. A total of 51% of the bilaterally transplanted glands incorporated and contained some cortical tissue at the time of removal; 64% of the unilaterally transplanted glands persisted under the same conditions. While there was considerable variation in the amount of cortical tissue in these seventy-four grafts, all of them contained some columns of cortical cells which appeared quite normal histologically (fig. 7). While cortical proliferation was not so extensive in grafts from normal hosts as in those from doubly suprarenalectomized animals, these experiments are regarded as sufficient to demonstrate conclusively that some cortical proliferation does occur in intra-ocular grafts in hosts possessing both intact suprarenal glands (compare figs. 2 and 3).

By reference to table 1 a, it may be seen that a lower percentage of bilateral transplants were recovered than unilateral ones. When observed through the corneas, the bilateral grafts appeared pallid and possessed relatively large necrotic centers which persisted longer than in the unilateral grafts. The unilateral grafts appeared to be more extensively vascularized and the necrotic centers were not often visible for longer than 10 days. Histological preparations revealed more extensive cortical proliferation in the unilateral than in the bilateral grafts.

From their studies on autotransplantation of suprarenal glands in suprarenalectomized hosts, Wyman and tum Suden ('32) reported a definite sex difference in the ability of the grafts to proliferate cortical columnus. During the present experiments no consistent sexual variation was observed grossly or histologically, although accurate measurements of

TABLE 1
Summary of incorporated suprarenal transplants¹

BILATERAL TRANSPLANTATION				UNILATERAL TRANSPLANTATION			
Type of host	Number of transplantations made	Number of grafts incorporated	Per cent incorporated	Type of host	Number of transplantations made	Number of grafts incorporated	Per cent incorporated
a. Entire glands to normal hosts							
Females	56	30	53.57	Females	27	18	66.66
Males	40	19	47.50	Males	12	7	58.33
Total	96	49	51.04	Total	39	25	64.09
b. Entire glands to doubly suprarenalectomized hosts							
Females	24	22	91.66	Females	12	12	100.00
Males	88	78	88.63	Males	36	33	91.66
Total	112	100	89.28	Total	48	45	93.75
c. Entire glands to normal hosts plus pituitary implants							
Females	30	22	73.33	Males	2	2	100.00
d. Capsules to doubly suprarenalectomized hosts							
Females	16	11	68.75	Males	4	4	100.00
e. Medullae to normal hosts							
Females	22	6	27.27	Females	5	0	00.
f. Medullae to doubly suprarenalectomized hosts							
Females	8	1	12.50	Females	5	0	00.

¹ Fibrotic or calcified nodules were not counted as incorporated grafts. The transplants removed during the first 10 days after insertion into the eyes are not included in the table.

the regenerated cortices were not made. A higher percentage of grafts were recovered from female hosts than from male hosts (table 1 a, b). This variation is suggestive but its significance is problematical.

Bilaterally suprarenalectomized hosts. One-hundred and twelve whole suprarenal glands from young donors were

transplanted to both eyes of fifty-six bilaterally suprarenal-ectomized hosts. Eighty-nine per cent of these grafts were recovered. Ninety-three per cent of the unilateral grafts were recovered following persistence in the anterior chambers of bilaterally suprarenalectomized animals (table 1 b). A significantly higher percentage of suprarenal transplants persisted in suprarenalectomized hosts than in normal animals (compare table 1 a, b). A slightly higher percentage of grafts were recovered when transplanted unilaterally than when transplanted bilaterally. Among the suprarenalectomized hosts, a slightly lower percentage of grafts were recoverable from the males than from the females.

All of the grafts in suprarenalectomized animals were conspicuously larger than those persisting in hosts with intact suprarenals (compare figs. 2 and 3). Contrasted with grafts in normal animals, they rapidly acquired a deep pinkish coloration due to vascularity. During the second and third days subsequent to insertion of the transplants, the necrotic centers were quite large but by the tenth day the necrotic areas were hardly visible, if present at all. This reduction of the necrotic centers indicated that the cortical columns were rapidly regenerated from the peripheral glomerulosa and capsule. Histological studies of the grafts from suprarenalectomized hosts indicated that cortical proliferation was very extensive as compared with either bilateral or unilateral grafts persisting in normal hosts. From the sectioned material it was not possible to detect any consistent difference in the degree of cortical proliferation in the grafts persisting bilaterally and unilaterally, nor in the grafts persisting in male and female hosts.

Normal hosts plus pituitary homoimplants. Fifteen normal female rats were bilaterally transplanted with suprarenal glands from 10-day-old donors, and to each host was administered one homopituitary implant at 48-hour intervals for a period of 1 month. Two normal male hosts were unilaterally transplanted and treated in the same manner. Seventy-three per cent of the bilaterally transplanted glands were recovered as well as the two unilaterally transplanted glands (table 1 c).

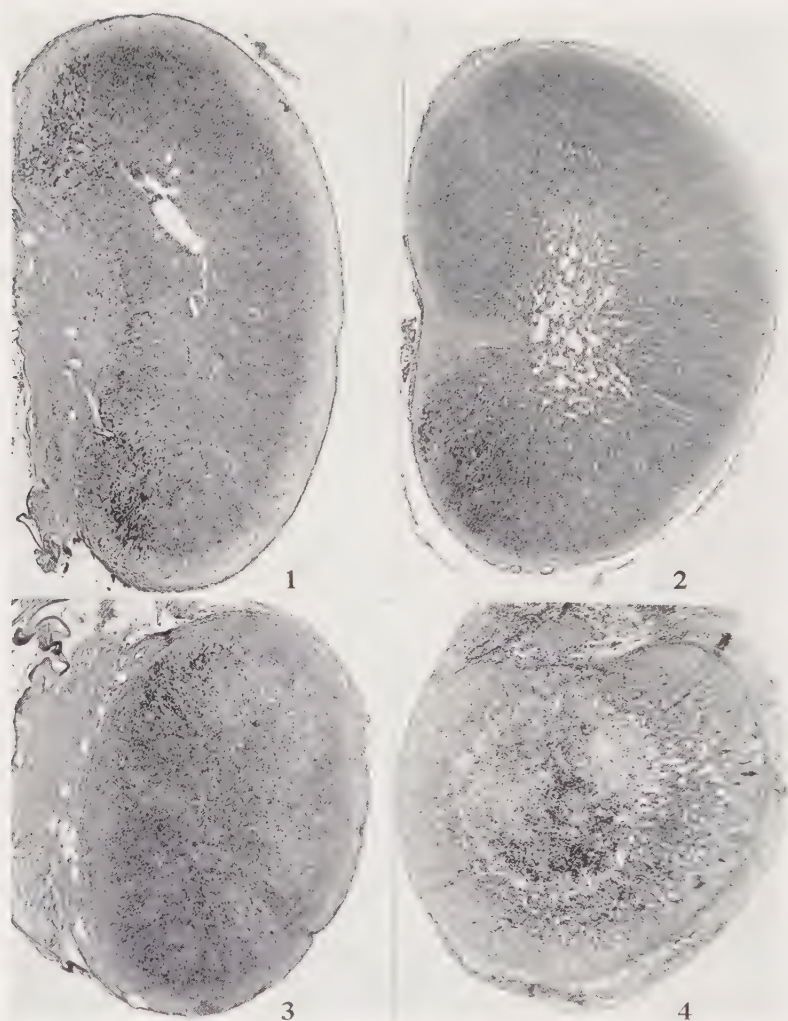


Fig. 1 Photomicrograph of a suprarrenal graft which regenerated from a transplanted capsule. The enucleated capsule was from a young female donor and was allowed to persist for 2 months in the anterior chamber of an adult supra-renalectomized female host. Bouin-Mallory. $\times 40$.

Fig. 2 Suprarrenal graft subsequent to persistence for 3 months in the eye of a supra-renalectomized female host. Section is through the widest diameter of the graft. Bouin-Mallory. $\times 35$.

Fig. 3 Comparable graft following persistence for 3 months in the eye of a normal female host. Section is through the widest diameter of the graft (compare with fig. 2). Bouin-Mallory. $\times 35$.

Fig. 4 A suprarrenal transplant after persistence for 4 days in the eye of a supra-renalectomized host. The necrotic center, regenerating cortical columns, and capsule are visible. Bouin-Mallory. $\times 35$.

The percentage of functional grafts recovered from normal hosts receiving pituitary implants was significantly greater than that recovered from comparable hosts which were not implanted (table 1 a, c). The size of the grafts from treated hosts, as well as the degree of cortical regeneration, did not equal that of grafts persisting in bilaterally adrenalectomized hosts but was much in excess of that characteristic for comparable untreated hosts.

Out of 243 recovered grafts of whole suprarenal glands it has been possible only in one case to identify persisting medullary tissue with the fixatives and stains here employed. This one instance was a gland from a newborn donor transplanted unilaterally to the anterior chamber of a bilaterally supra-renalectomized male (fig. 6).

II. Homotransplantation of incomplete glands

Transplantation of enucleated capsules. Both adrenal glands were removed from 22-day-old female donors, placed in Ringer's solution, and the tips of the glands severed in order to express the medulla and most of the cortex from the capsules. These enucleated capsules, with some adherent glomerulosa, were transplanted bilaterally to the eyes of eight supra-renalectomized female hosts and unilaterally to four supra-renalectomized male hosts. The transplanted capsules were removed for histological and cytological studies after persistence for approximately 2 months. Sixty-eight per cent of the bilateral grafts and all of the unilateral grafts were recovered and found to contain apparently functional columns of cortical cells (table 1 d and fig. 1). Cortical restoration from such transplanted capsules seemed to be as extensive as in whole transplants persisting in hosts of this category. This verifies the earlier observations of Ingle and Higgins ('38) who observed cortical replacement in enucleated suprarenal capsules sutured to the ovaries of rats.

Transplantation of expressed medullary tissue. All workers agree that the medullary portion of the suprarenal gland, unlike the cortex, very seldom persists in transplants. How-

ever, Wyman ('28) identified medullary tissue in two suprarenal grafts. In the present experiments an attempt was made to analyze some of the factors which condition the incorporation of the suprarenal medulla. Medullary tissue was expressed from the glands of 20-day-old donors, and while the glands were in Ringer's solution the adherent cortex was further teased off under a dissecting microscope. These teased medullae were transplanted bilaterally and unilaterally to the anterior chambers of normal and suprarenalectomized female hosts. None of the unilateral transplants persisted. Twenty-seven per cent of the medullary transplants to normal hosts and 12% of the transplants to suprarenalectomized hosts were recovered after persistence for from 7 to 20 days (table 1 e, f). These recovered grafts underwent little increase in size after introduction into the eyes and consisted of small strands and cords of medullary cells encapsulated by connective tissue. In the absence of the suprarenal capsule, cortical replacement did not occur and this necessitated autopsy as the animals showed symptoms of cortical insufficiency.

III. Cortical replacement in suprarenal homotransplants

General histology. A series of suprarenal transplants were removed at 12-hour intervals subsequent to insertion into the anterior chamber in order to study the early degenerative changes and the proliferation of new cortex in both normal and doubly suprarenalectomized hosts. By 12 hours after introduction of the transplant, the medulla and the fasciculate zone of the cortex had begun to deteriorate, and by 24 hours necrotic changes had extended peripherally to such extent that only the capsule and outer portion of the zona glomerulosa contained viable suprarenal cells. By 48 hours, the capsule and remaining portion of the cortex had become canaliculized by blood vessels, the necrotic center consisting of a reticular framework in the meshes of which macrophages, polymorphonuclear leucocytes, lymphocytes and extravasated erythrocytes were identifiable. Large hemorrhagic areas were present in the necrotic zone by 60 hours, and vascularization of the outer portion of the transplant had increased. The

extent of this initial degeneration did not differ appreciably in transplants recovered from normal and suprarenalectomized hosts.

Soon after the establishment of blood vessels the proliferation of cortical cells ensued rapidly (fig. 4). Histological observations on these early transplants demonstrated clearly that cortical restoration proceeds much more rapidly in suprarenalectomized animals than in the presence of the host's suprarenal glands. In the transplants from suprarenalectomized hosts, centers of active mitotic proliferation in the capsule and peripheral rim of glomerulosa cells were conspicuous by the end of 48 hours subsequent to insertion of the glands (fig. 5). During the first 10 days, mitoses were much less frequent in transplants from normal hosts than in comparable grafts recovered from suprarenalectomized animals.

Proliferation from these areas produces columns of cortical cells which are pushed inwardly and encroach upon the necrotic center. By 3 weeks, the volume of proliferated cortex in grafts from suprarenalectomized hosts often approximated that present in the intact glands of normal animals. The regenerated cortical substance consists of radially disposed cords between which are numerous capillary blood vessels. Although zonation is relatively indistinct in the suprarenal gland of the rat, the cellular arrangement of the cortical cells in transplants seems to be very similar to that of normal intact glands (fig. 7). In well-incorporated grafts, the zona glomerulosa is represented by irregular loops and masses of small cells next to the capsule; the zona fasciculata by the straight and radially arranged cords; and the zona reticularis by the open, anastomosing cords characterized by cell fragmentation and destruction.

*Cytological observations.*² The observations of Zwemer ('36) and Ingle and Higgins ('38), in addition to those here-

² The cytological observations on transplants have been supplemented by comparable studies of normal, intact suprarenal glands of mice, bats and rats. Observations on the normal cytology of the suprarenal glands of the bat will be published jointly by Mary J. Guthrie and the present author.

with reported, indicate that the capsule is the source of cortical cells in both intact and transplanted glands. With the exception of a delicate reticular background and a few invading cells of vascular and connective tissue origins, only one type of cortical cell is identifiable. In the cortices of intact glands, as well as in incorporated and functional grafts, there

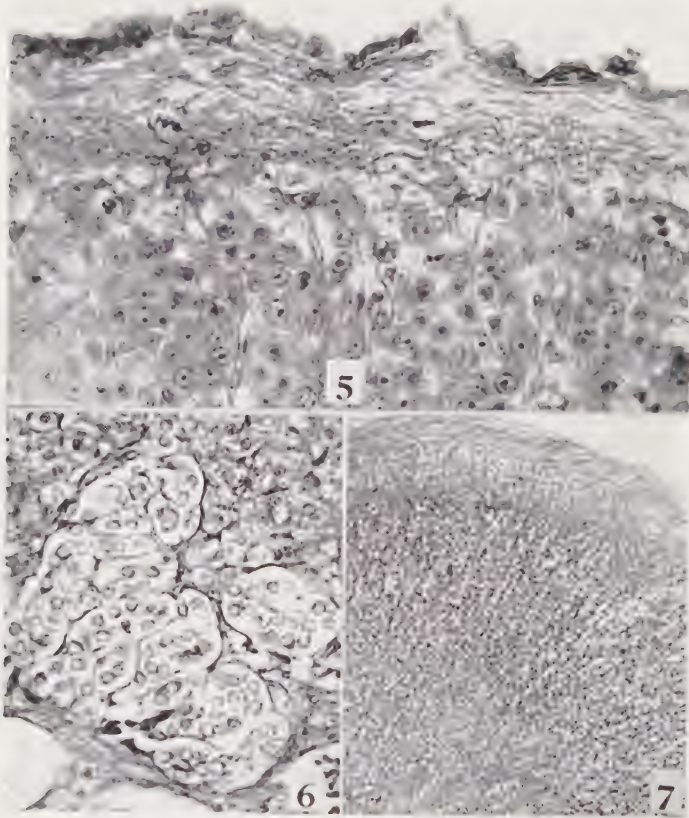


Fig. 5 Section of a suprarenal transplant which had persisted for 4 days in the anterior chamber of a suprarenalectomized host. Mitotic divisions are visible in the capsule. Bouin-Mallory. $\times 434$.

Fig. 6 Medullary cells from a whole gland transplanted unilaterally to the anterior chamber of a suprarenalectomized male host. Ehrlich's hematoxylin and eosin. $\times 267$.

Fig. 7 Portion of a suprarenal graft following persistence for 4 months in the anterior chamber of a normal female host. The capsule, zona glomerulosa, and radial cords of the fasciculata are visible. Bouin-Mallory. $\times 50$.

is apparently a continuous replacement of disintegrating, post-secretory cells by proliferation from the capsule.

The embryonic cells of capsular origin are small and possess relatively little cytoplasm. They are rounded or spindle-shaped and are frequently compressed against the fibers of the capsule. The cytoplasm of these undifferentiated cells contains a few granular chondriosomes. The nucleus is highly chromatic and contains one or more nucleoli. In transplants, these cells frequently undergo mitoses even before leaving the capsule.

The unspecialized cells of capsular origin are gradually pushed inwardly from the capsule and enter upon a period of growth and storage. The cytoplasm enlarges proportionately more than the nucleus. With the growth of the cell there is a tremendous increase in the number of chondriosomes which are either in the form of spheres or rods. In transplants fixed in Champy's fluid, some of these cells are found to contain many large osmophilic droplets. In general, the zona glomerulosa³ seems to be characterized by this type of cell. Although these growing cells are more differentiated than those of the capsule, the capacity for mitotic division has not been lost by all of them.

The fully differentiated cells, constituting the outer portion of the zona fasciculata, are the largest of all normal, pre-secretory, cortical cells. While the nuclei are only slightly larger than those of the embryonic cells, the cytosome has increased many fold. Due to the manner of cell renewal from the periphery and possibly to the restraining influence of the reticular meshwork and blood capillaries of the cortex, these cells are often compressed into rectangular shapes. In addition to varying numbers of finely-divided osmophilic droplets, the differentiated cells contain many fuchsinophilic spheres.

³Since the inward differentiation of cortical cells is a gradual process, it is believed that the conventional division of the cortex into glomerulosa, fasciculata and reticularis is of little significance. The terms are employed here as a method of conveniently locating general areas of the cortex.

Chondriosomes are present throughout the cytosome; between the fuchsinophilic and osmophilic granules. Cells at this stage are regarded as loaded cells of a secretory nature, but it has not been determined whether the osmophilic or the fuchsinophilic granules represent the actual precursor of the secretion; or, if both types of granules are precursors, which is the later stage.

After the cells discharge their secretory granules, the volume of the cytoplasm is diminished and only a few osmophilic and fuchsinophilic granules remain. Eventually, cytoplasmic vacuolation begins, the nucleus becomes pyknotic, and the cell appears inflated and rounded or ovate in outline. These cells undergoing early stages of post-secretory degeneration are particularly prevalent in the fasciculata, although they may be found in the reticularis. Cells of this type are regarded as the 'light cells' of other investigators. During later stages of degeneration, the cells decrease in size and are compressed by their neighbors into angular configurations. After fixation in Champy's fluid the cytoplasm of such cells stains intensely with acid fuchsin while the pyknotic nuclei are often stained by the aurantia. They are frequent in the inner half of the fasciculata and throughout the reticularis. Cells in such advanced stages of degeneration have been called 'dark cells' by recent investigators and 'siderophile cells' by earlier ones. In the reticular zone, these cells fragment and are apparently removed by phagocytes.

DISCUSSION

It is thought that nutritive conditions in the anterior chamber of the eye, as well as the lower temperature of this site (Turner, '38), explains in part, at least, why intra-ocular suprarenal transplants survive in normal hosts where there is no apparent deficiency of cortical hormone, but do not survive according to Wyman and tum Suden ('32) and Ingle and Higgins ('38) when transplanted to intraperitoneal and intramuscular positions in normal hosts. Notwithstanding the advantageous nutritional conditions in the anterior chamber

the more differentiated cells of the reticularis and fasciculata degenerated, the new cortex being formed by proliferation from the relatively undifferentiated cells of the glomerulosa and capsule.

With the exception of one instance out of 243 grafts recovered from the eyes following insertion of the whole glands, the medullary portions did not persist. The medulla seems to be as highly differentiated as the most specialized cells of the inner cortex and hence is not able to survive following a prolonged interruption of the vascular supply. Since the medulla apparently does not contain a reserve of embryonic cells, it is not replaced following the necrosis of its specialized elements. Even when the whole suprarenal gland is surrounded by a nutritional fluid, as when inserted into the anterior chamber of the eye, the centrally placed medulla probably does not receive by diffusion sufficient metabolites nor possess a satisfactory means of eliminating its metabolic wastes to prevent degeneration before vascularity is established. The observations reported herewith demonstrate that the frequency of medullary persistence is greatly enhanced by teasing away the surrounding cortices before introduction into the aqueous humor (table 1 e, f). It is believed that the medulla is a relatively specialized tissue but the present observations show that it may be transplanted successfully when provided with proper nutritional conditions. Wyman ('28) found that medullary cells survived in two of his autoplasmic grafts persisting in intramuscular sites. It should be noted that Wyman employed the technique of Jaffe and Plavska ('26). Hence the glands were bisected previous to introduction into the host thus making it probable that the medullary cells were near the periphery of the transplant where vascularization could ensue quickly. Since the medulla infrequently persists in whole suprarenal grafts as well as in normal hosts with intact cortices, there is no evidence indicating that the secretion of one part of the gland (cortex or medulla) is antagonistic to the incorporation of the other.

Some of the data collected in this series of experiments seems to verify and extend earlier observations of other workers indicating that some humoral agent in the organism regulates the incorporation and persistence of suprarenal transplants. Wyman and tum Suden ('32) reported that autoplasmic and homoplasmic transplants of suprarenal cortices do not persist in intramuscular sites in the presence of one or both of the animal's own glands. They also found that the degree of cortical proliferation occurring from a transplanted fragment depended upon the number of fragments which persisted. This limitation was interpreted in terms of Halsted's law of deficiency. In 1937, Wyman and tum Suden found that autoplasmic and homoplasmic suprarenal transplants did not persist in rats which had been suprarenalectomized and hypophysectomized, and concluded that the cortico-adrenotropic hormone of the pars distalis of the hypophysis conditioned the persistence and differentiation of cortical grafts. Numerous investigators (e.g., Emery and Atwell, '33; Selye and Collip, '36; and Atwell, '37) seem to have established that the cortico-adrenotropic principle of the anterior lobe normally regulates the intact suprarenal glands. Ingle and Higgins ('38) have come to similar conclusions as the result of their studies on suprarenal transplantation. In general, our own experiments indicate that there is a physiologic factor in the organism which conditions the incorporation and differentiation of the cortices, and we believe this to be the cortico-adrenotropic hormone of the pars distalis. Such a concept is based upon the fact that the extent of cortical proliferation in suprarenal grafts, as well as their rate of persistence, seems to be inversely proportional to the amount of such tissue present in the organism. The rate of incorporation and the degree of cellular proliferation in the cortical transplants were increased by bilateral suprarenalectomy or by the administration of pituitary implants to normal hosts. Furthermore, more cortical regeneration and a higher rate of persistence occurred subsequent to unilateral transplantation than following bilateral transplantation. Within the

limits of these experiments, there is no evidence that the incorporation of medullary tissue is conditioned by the adrenotropic principle since the rate of survival of the teased medullae was not augmented by double suprarenalectomy of the hosts.

It is conclusively demonstrated that intra-ocular homotransplants of the cortices persist and undergo some degree of regeneration in the presence of both intact glands of the host in addition to one entire suprarenal graft. Therefore, if cortical proliferation is dependent upon cortico-adrenotropic hormone, it is necessary to postulate 1) that there is more hormone present in the organism than is needed to maintain the intact suprarenal glands, or 2) that there is only enough of the cortico-adrenotropic principle present to support the intact glands but no 'preference' is shown the intact glands over the transplants or 3) that the cortical cells possess an inherent ability to differentiate in the absence of the cortico-adrenotropic hormone. Suprarenal glands are not peculiar in this respect. In earlier studies, it was demonstrated that some of the homotransplanted testes from prepuberal donors incorporated and proliferated mature spermatozoa in the eyes of hosts possessing both scrotal testes (Turner, '38). Whether the intact set of suprarenal glands, or testes, is damaged by the maintenance of a transplanted set of such organs remains obscure. The fact that some of the endocrine tissues may be cultured *in vitro* indicates that they possess some ability to grow in the absence of hypophyseal or other glandular principles (Lux, Higgins and Mann, '37 a, b).

On the basis of evidence obtained from the cytological studies of normal and transplanted suprarenal cortices it is believed that the zonation of the gland is due to the accumulation of cells in a comparable state of differentiation or post-secretory degeneration. That the cortical cells are proliferated from the peripheral glomerulosa and capsule and differentiate as they are pushed inwardly seems to be thoroughly established by the contributions of Mulon ('03), Goormaghtigh ('22), Hoerr ('31), Zwemer ('36) et al. This concept has been

based on three facts: 1) the prevalence of mitoses in the outer portion of the cortex, 2) histological evidence of cell destruction and removal in the reticular zone and 3) the degeneration of the inner cortex in transplants and subsequent restoration from the periphery. It was thought that the problem of cortical cell differentiation and destruction in both intact and transplanted glands could be elucidated further by the employment of cytological techniques.

The cortical cells differ with respect to size, nucleoplasmic ratio, abundance of chondriosomes, chromaticity of the nucleus, vacuolation of the cytosome, and the presence of lipoids and fuchsinophilic globules. The cytologically different cells of the cortex are interpreted as representing stages in a series of differentiatinal changes through which all of the cells eventually pass. There is no evidence that the cells of the cortex undergo more than one secretory cycle. From cytological studies on differentiating oocytes and developing eggs, Guthrie ('25 and '29) pointed out that the visible inclusions of the cytoplasm provided reliable criteria for estimating the stage of growth and differentiation of particular cells. This concept has been utilized in the present seriation of the cortical cells.

The embryonic or totipotent cells of the capsule or adjacent to it are typical of such cells wherever found in animal tissues since they possess scant cytoplasm, few chondriosomes, no secretory granules, and little if any stored lipid. The growing cells of the zona glomerulosa are characterized by more abundant cytoplasm containing varying amounts of stored lipid in the form of relatively large globules. As differentiation progresses pressure exerted at the periphery causes the fasciculata cells to become rectangular or crescentic in shape. The cytoplasm increases markedly in volume and becomes filled with fuchsinophilic granules and relatively small droplets of emulsified lipoids. These cells (spongicytes) are regarded as highly differentiated and secretory, but it is not possible to indicate what relationship the fuchsinophilic and osmophilic granules bear to the actual secretion. After

discharge of the secretion the cells become smaller and apparently may persist for an indefinite period before they degenerate sufficiently to be eliminated by the circulation. The 'light' and 'dark' cells are thought to be stages in post-secretory degeneration.

SUMMARY AND CONCLUSIONS

1. Both bilateral and unilateral homotransplantation of suprarenal cortices is possible in the anterior chamber of the eyes of normal rats. The extent of cortical regeneration in suprarenal grafts as well as the frequency of persistence is augmented by suprarenalectomy and by homopituitary implantation. The evidence seems to indicate that differentiation in cortical transplants is conditioned by the cortico-adrenotropic principle of the anterior hypophysis.

2. The medullary tissue is relatively specialized but may persist in homoplastic grafts if the period of nutritional interruption is minimized.

3. The suprarenal cortex contains a single type of secretory cell which is present in varying stages of growth, differentiation, and post-secretory degeneration.

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SEPARATION OF THE ACIDOPHILIC ELEMENTS OF THE TISSUES INTO TWO GROUPS

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The profoundly differential staining reactions obtained by the use of phosphotungstic and phosphomolybdic acid in connection with certain acid dyes have long been known. As applied for example in the well-known Mallory's connective tissue stain, the method serves to divide the cytoplasmic elements of mammalian tissue into two major groups according to their staining reactions. It consists in staining Zenker-fixed material in acid fuchsin, which confers a more or less uniform color upon the tissue. Sections are transferred to phosphomolybdic or phosphotungstic acid for selective differentiation. Certain tissue elements, relatively acidophilic, including general cytoplasm, muscle, erythrocytes, acidophil cells of the pituitary, zymogen (which we may call class 1) retain the acid fuchsin; others, including collagen, reticulum, cartilage, mucin, basophil cells of the pituitary (class 2), are completely decolorized. Since it is desirable that these elements have color, a secondary dye, anilin blue, is used for that purpose. Various modifications of Mallory's original stain employing other fixatives and dyes, when intelligently performed and microscopically controlled, yield similar results with respect to the staining reactions briefly described above.

Orange G may be incorporated with the secondary dye, anilin blue (Mallory, '00) or with the primary dye, acid fuchsin (Crossmon, '37). If material is fixed in Zenker's or a bichromate fixative, erythrocytes possess a greater affinity for orange G than acid fuchsin and consequently are

stained orange. The application of orange G has however no bearing on the following discussion, and no further reference need be made to this component of Mallory's stain.

The specific action of phosphotungstic acid upon certain elements of the tissues is well illustrated by the use of Congo red. The dye acid is blue, but its sodium salt is red. The red color of the salt is readily changed by weak acids into blue. If sections are stained with a saturated solution of this dye containing a small amount of sodium chloride, the tissue is quite homogeneously stained red. Sections are transferred to 2% phosphotungstic acid for a few seconds, quickly rinsed in distilled water, and examined under the microscope. Tissue elements of class 1 are red, those of class 2 are blue. Phosphotungstic acid in aqueous solution is unable to remove this dye selectively from the secondary elements yet expresses its specificity by changing their color to blue. The result is a Mallory stain with the employment of one dye. Permanent preparations in xylol balsam are however not possible because the blue coloration of the secondary elements is gradually discharged in ethyl alcohol.

Examination of sections stained by Mallory's original method shows that certain components forming class 1 are more intensely stained than others, because these more acidophil elements have a greater affinity for acid fuchsin. This is also true when certain other dyes are used as the primary stain, e.g., ponceau de xylinine (Masson, '29). Preparations stained with hematoxylin and eosin show this same tinctorial differentiation. A few of these elements long since observed to have greater affinity for eosin and consequently to be more intensely stained by it have been termed eosinophilic, or more generally, acidophilic or oxyphilic.

It occurred to the writer that with appropriate technique and choice of dyes these more intensely staining acidophil elements of class 1 might be more sharply differentiated.

Experiment with various acid dyes showed that they may be divided into three groups according to their reactions with phosphotungstic acid.

1. Dyes that show no reaction to phosphotungstic acid. Mercurochrome is the only example available at this time.

2. Dyes such as acid fuchsin or ponceau de xyline that may be used for the division of histological elements into two major classes.

3. Dyes that have affinity for only the strongly acidophil elements of protoplasm. The fluorescein dyes eosin Y, erythrosin, phloxine and rose bengal are in this category. Chromotrope 2R, an azo dye closely related to orange G, is also in this group, but its use has not yet been fully investigated.

With this information, the following modification of Mallory's stain, employing the fluorescein dyes in place of acid fuchsin, has been devised.

1. Fixation. The usual methods of fixation may be employed. Bichromate-formalin (or Zenker-formalin) is essential for the preservation of zymogen granules or other histological elements soluble in acid fixatives. Bouin's fluid is useful for the demonstration of erythrocytes and other acidophilic elements not soluble in acid fixatives, and when used particularly for erythrocytes has the advantage that many other strongly stainable small elements (e.g., zymogen granules) are not preserved. Neutral 10% formalin containing 0.85 gm. of sodium chloride for each 100 cc. is also a suitable general fixative.

The fixing fluids should be prepared and used exactly as follows.

Bichromate formalin (proportions as used by Masson, '29)

3% potassium bichromate in distilled water 90 cc.

Commercial formalin (neutralized) 10 cc.

Commercial formalin may be neutralized by the addition of marble chips a few days prior to fixation. The mixture is relatively unstable and should be prepared immediately before use.

The duration of fixation is 18 to 24 hours, according to the size of material. Pieces should not be greater than 8 mm. in

thickness; their other dimensions are of no importance. The quantity of fixing fluid should be at least fifty times the volume of the tissue. Precipitation gradually occurs in the bichromate formalin mixture after a few hours. Pieces should be transferred to freshly prepared fixative. One or two changes are usually sufficient.

Following fixation, material is washed in running tap water for 18 to 24 hours and transferred to 70% ethyl alcohol, preliminary to further dehydration.

Bovin's fluid

Picric acid, saturated aqueous solution	75 cc.
Commercial formalin	20 cc.
Glacial acetic acid	5 cc.

This mixture is stable and may be prepared in large quantities. The size of pieces and time of fixation are as for bichromate formalin.

Following fixation, material is washed in 70% ethyl alcohol. Several changes should be used to remove excess picric acid.

2. Dehydrate completely, embed in paraffin, cut and mount as usual.

3. Stain in Mayer's acid hemalum (standard formula as given in Lee's Vade-Mecum). The nuclei should be slightly overstained.

4. Wash sections in running tap water for at least 15 minutes followed by a change to distilled water.

5. Stain in thymol erythrosin.

Erythrosin, bluish (National Aniline Co.)	2.5 gm.
Thymol crystal	0.1 gm.
Distilled water	500 cc.

Heat gently until thymol and erythrosin are dissolved. The solution should be filtered, as a slight precipitate usually results. The addition of thymol is not necessary but of value because it increases the intensity of staining. In this laboratory it is always used with eosin Y whenever that dye is used as an acid counterstain, for example with hematoxylin.

Other fluorescein dyes including eosin Y, phloxine and rose bengal may be substituted for erythrosin in the formula above. As stated above, mercurochrome is an exception; phosphotungstic acid shows no selective action with reference to this fluorescein dye.

A suggested staining time is 20 minutes. Somewhat more intense results may be obtained by staining for a longer interval.

6. Rinse in distilled water. This step is sometimes omitted. It is usually necessary if the sections contain striated muscle.

7. Transfer to 2% phosphotungstic acid in distilled water. Sections are left in this reagent 15 to 20 minutes or until the elements of class 2 are microscopically orange, components in class 1 remaining red. Like Congo red, erythrosin is an indicator. Phosphotungstic acid in aqueous solution is unable to remove the dye from components of class 2 yet expresses its specificity by changing their color.

For best results fresh phosphotungstic acid should be used for every second rack of slides.

8. Rinse in distilled water. This step is sometimes omitted. It is usually necessary if sections contain striated muscle.

9. Transfer to absolute ethyl alcohol. Sections should be gently agitated to allow for uniform and rapid decolorization. Elements of class 2, colored orange in step 7, are immediately decolorized. Smooth muscle, skeletal muscle and general cytoplasm, colored red in step 7, require a longer time. As a final result, erythrosin is removed from all tissue elements with the exception of those histological structures that are strongly acidophil.

Sometimes a little difficulty may be experienced in removing all dye from muscle substance, especially with thick or torn sections. If this occurs, slides are transferred from the absolute alcohol back to phosphotungstic acid for an additional time interval.

At this stage the strongly acidophilic elements are red, the less strongly acidophilic and the basophilic elements are colorless, and the nuclei have been stained by the hematoxylin.

The procedure may be terminated by completing dehydration in one or more changes of absolute alcohol, followed by clearing in xylol and mounting in xylol balsam.

It will however usually be desirable to introduce color into the elements left colorless by the preceding steps. Proceed as follows.

10. Remordant in 2% phosphotungstic acid (repeating step 7) for 2 to 3 minutes.

11. Transfer to light green (or anilin blue).

Light green (Krall Microcolor; Eimer and Amend)	1 gm.
Distilled water	100 cc.
Glacial acetic acid	1 cc.

Since it is difficult to examine the preparation in this stain, the suggested time is 2 to 4 minutes. Light green and anilin blue are mordanted to the elements of class 2. With additional staining time the decolorized elements of class 1 may be lightly stained.

The use of light green is suggested for most preparations. For the demonstration of the basophil cells of the pituitary, mucin and certain other basophil structures, anilin blue is superior. The formula is:

Anilin blue (National Aniline Co.)	2 gm.
Distilled water	100 cc.
Glacial acetic acid	2 cc.

The procedure is practically the same as with light green, except that sections are left comparatively longer in the glacial acetic acid (step 13) to obtain optimum differentiation.

12. Rinse in distilled water, removing the sections immediately.

13. Transfer to 1% glacial acetic acid to remove the light green or anilin blue loosely bound to other than elements of class 2. Sections should be left in the acid until that result is attained. Inspection of a trial slide with the microscope will determine optimum differentiation. Thirty to 60 seconds is usually sufficient for light green.

The final result should be as indicated in the table at the end of this paper. Those elements listed in class 1A still retain the erythrosin or other substituted fluorescein dye. Those listed as class 1B, having little affinity for either primary or secondary dye are colorless or very lightly stained by the light green or anilin blue.

14. Rinse in distilled water removing the sections immediately.

15. Pass through two or three changes of absolute ethyl alcohol followed by three changes of xylol. Mount in balsam.

DISCUSSION

Results obtained, at this time of writing, with respect to color are listed in the following table.

<i>Class 1</i>		<i>Class 2</i>
<i>A</i>	<i>B</i>	
<i>Red</i>	<i>Colorless or very lightly stained green or blue</i>	<i>Green or blue</i>
Erythrocytes	General cytoplasm	Collagenous fibers
Eosinophil granules of leucocytes	Smooth muscle	Reticulum
Certain zymogen granules	Skeletal muscle	Cartilage
Russell bodies of carcinoma, sarcoma		Bone
Acidophil cells of the pituitary		Dentine
Keratinized epithelium		Basophil cells of pituitary

The staining reactions of the components of class 2 will be well known to the reader through the use of the original Mallory's stain. The separation into two classes, of the elements staining with the acid fuchsin in the original Mallory's stain, which is achieved by the new technique herewith described, will require further study. Many questions suggest themselves as to chemical reasons for the high affinity for acid dyes shown by the elements of class 1A, and also as to possible relationship (or lack of relation) between these elements, seemingly so diverse.

Specific applications are also a question for additional consideration. Other workers may find that the substitution of other fluorescein dyes or other methods of fixation serve better a particular purpose. The time of staining or decolorization should be considered as open to modification to suit specific cases.

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REPRODUCTION IN AMERICAN MONKEYS

I. ESTROUS CYCLE, OVULATION AND MENSTRUATION IN CEBUS ¹

G. W. D. HAMLETT ²

ONE FIGURE

INTRODUCTION

Our knowledge of the physiology of reproduction in monkeys has been based almost entirely upon observations of the Old World monkeys and apes; the platyrrhine or American monkeys having been largely neglected. I know of only two articles which deal with the latter in anything more than a superficial fashion. The first of these is in Rengger's 'Säugethiere von Paraguay,' published in 1831, in which Rengger details his observations upon *Cebus azarae* in the field and in captivity, and states that the female has a menstrual bleeding; that he should have thought of this as a sign of estrous is of course to be expected when we consider the state of our misinformation 100 years ago upon primate physiology, and does not detract from the value of his

¹ These studies on *Cebus* were undertaken as part of a 2-year program for the study of the reproductive behavior and embryology of South American mammals, planned under a Fellowship from the John Simon Guggenheim Memorial Foundation. This article presents the first results obtained in the Fellowship program. Other material and observations will be described in later papers. The monkey studies were aided by a Grant from the Committee on Sex Research of the Rockefeller Foundation.

The work on *Cebus* was done while a guest of the Rockefeller Foundation at the Epidemiological Laboratory of the Yellow Fever Service in Anapolis, Goyaz. To Drs. Fred L. Soper and D. B. Wilson, Director and Assistant Director of the Foundation in Rio de Janeiro, and to Drs. Clark L. Yeager and Gastão Cesar, successively Directors of the Anapolis Laboratory, I tender my sincerest thanks for the facilities afforded me and for the many other kindnesses shown me during my stay in Brazil.

² Guggenheim Fellow, 1936-1938.

pioneering work. The second paper to which I refer is that of Goodman and Wislocki ('35) dealing with the vaginal cycle of a spider monkey, *Ateles*. These authors found in their animal a regular vaginal cycle of about 28 days, with menstruation during the low part of the cycle and ovulation approximately midway in the intermenstrual period.

This article presents a series of observations upon the species of cebus monkey found in Southern Goyaz, Brazil. I have been able to amplify Rengger's findings and to correlate both his and mine with our modern conceptions of primate reproduction; furthermore, *Cebus* proves to differ sufficiently from *Ateles* and the Old World primates to lend an added value to its study from the comparative standpoint. The species which was under observation has not yet been determined taxonomically with any certainty; in the present chaotic state of the systematics of *Cebus* an accurate identification must probably wait on the long overdue revision of the genus. However, it is certainly closely related to *Cebus azarae*, if indeed it does not eventually prove to fall within the range of variability of that species. Specimens in the South American museums, coming from the same general locality and agreeing with my animals in appearance, are variously labeled *Cebus apella*, *azarae*, *cirrifer*, and *fatuellus*.

Some twenty-odd animals were available for the study, not all of these however being usable. After the young and those obviously ailing were eliminated, five of the most vigorous appearing females were given the run of a large outdoor cage together with an adult male. Some substitutions were made during the course of the observations; in all, seven females were studied for longer or shorter periods. In the beginning, smaller portable cages were tried; these proved unusable, as all signs of cyclic activity disappeared within 2 weeks under these conditions. It took about 3 weeks for the cycles to become reestablished after return to the large cage. The percentage of cellular sediment in the vaginal lavage, using the technique worked out by Hartman for the macaque, was recorded daily for each animal under

observation. Laparotomies were performed on various occasions, to correlate the ovarian conditions with the vaginal cycle or to explore for a suspected ovum, and at the conclusion of the experiments the reproductive organs were removed for study.

VAGINAL CYCLES

The criterion of cyclic reproductive activity used in the experiment was, as already stated, Hartman's method of measuring the percentage of vaginal sediment. The vagina was washed out daily, in each animal, with a pipette-full of water, the washing collected in a test tube of appropriate size and allowed to stand until all cellular debris had settled to the bottom, the percentage of debris in the washing being then recorded. A drop of the sediment was usually placed under the microscope so as to observe changes in the relative numbers of nucleated epithelial cells, cornified squamous cells, and leucocytes present in the lavage. Hartman has shown that the amount of vaginal sediment furnishes an accurate index to the condition of ovaries and uterus in the macaque; and a whole host of workers have shown, in species from rats to women, a close correlation between the kinds of cells present in the vagina and the phases of the ovarian and uterine cycles. It thus seemed logical to hope that in the cebus monkey also this simple method might serve as an indicator of the changes in the internal reproductive organs.

Within a few days after beginning the records of lavages it was seen, in fact, that most adult females were showing variations, usually cyclic, in the amount of vaginal debris. The regularity of the cycles seems to depend upon the health of the individual; I have already pointed out that confinement in small cages suppressed the cycles, and animals in poor condition were irregular or showed a constantly low percentage of sediment. On the other hand, two especially active and vigorous females, nos. 991 and 992, showed exceptionally regular and well-marked changes in the vaginal washings.

These two were the healthiest animals of all those used, both actually gaining in weight during the experiment in spite of daily handlings and several laparotomies. I believe that these two animals represent the normal for the species in nature, I am consequently presenting their records in detail (fig. 1) and shall draw largely upon them during my discussion.

Form of curve

The form of the curve which shows the percentage of vaginal sediment may be seen in the figure. In the low part of the cycle the vaginal washings showed usually less than 10% of cellular debris, sometimes only a bare trace in a practically clear lavage. Menstruation, when it occurred, took place during this low phase. Following a few days of oscillations at a low level, the amount of sediment would begin to increase, reaching a height of between 40 and 65%. The peak of the curve was usually quite sharp, and was followed by an abrupt drop to the low premenstrual level. The graphs show on several occasions a drop in the curve on the day when copulation took place; this drop is only apparent, and is due to unavoidable erroneous measurement of the vaginal sediment following insemination. The ejaculate formed a vaginal plug which persisted for hours as a transparent, celloidin-like mass so filling the vagina that it was almost impossible to get anything in the lavage other than fragments of the plug. Comparison of the copulatory and non-copulatory cycles indicates that readings for the day of copulation may be too low by as much as 30% of the total lavage.

A feature of the curve which may prove to be of importance is the presence in many cycles of a sharp dip which breaks the postmenstrual rise to the ovulatory peak. This notch is seen in four out of 7 times in the curve for 991, and six out of eight times in that of 992. The significance of this is not apparent in our present state of knowledge. Dr. J. E. Markee tells me that there is a similar temporary check in the cyclic growth of intra-ocular endometrial transplants in

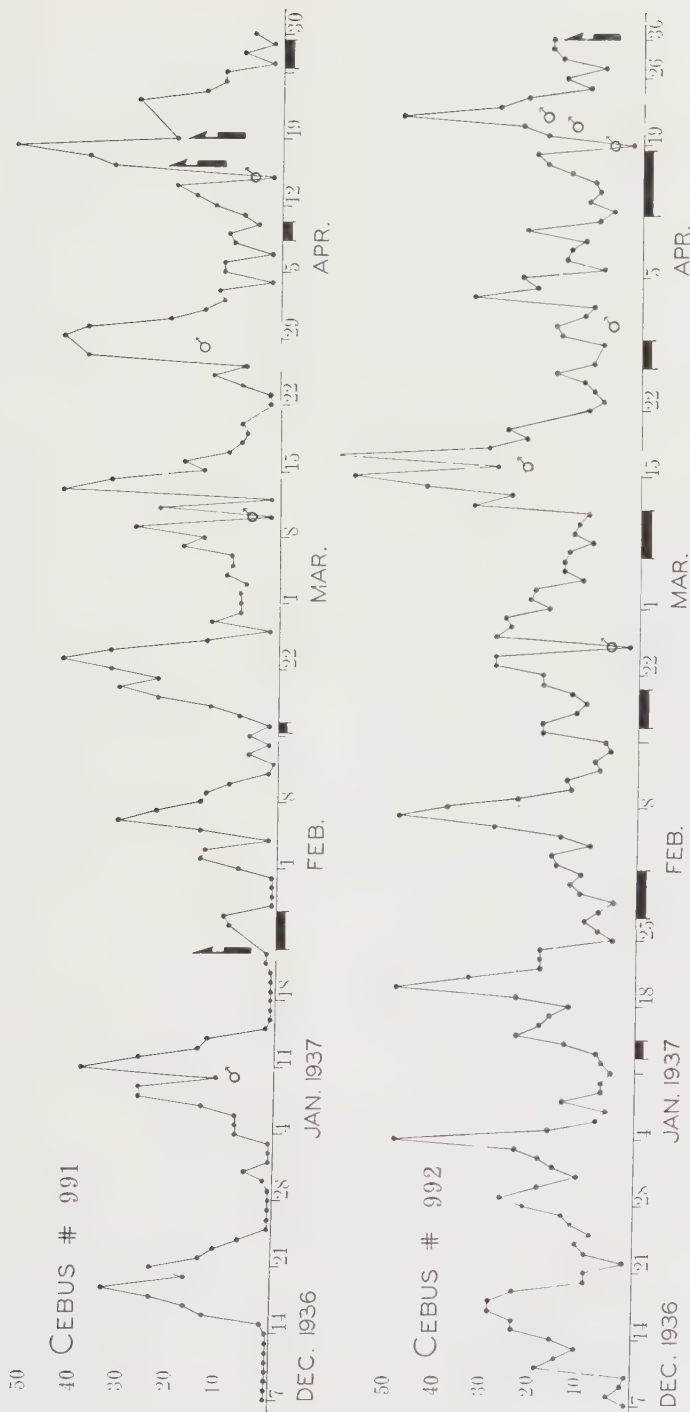


Fig. 1 Vaginal desquamation curves of Cebus females nos. 991 and 992. Each dot represents 1 day's reading. Height of the dots above the base line indicates the amount of sediment in the lavage. This may be read off on the scale at the left, which shows the percentage. The base line is marked off in weekly intervals. Menstruation is indicated in heavy black on the base line. Copulation is shown by the male symbol, ♂. The conventionalized scalpel sign indicates a laparotomy.

his rhesus monkeys; this occurs in both ovulatory and non-ovulatory cycles. An apparently identical irregularity is found in the graphs showing cyclic perineal swelling in the baboon, according to Gillman ('35). Gillman believes that this dip in the curve, occurring 24 to 72 hours before the maximum swelling, may be due to ovulation. Whatever may be true of the baboon, I am certain that in *Cebus* the notch in the vaginal curve is pre-ovulatory, probably by 48 to 72 hours. Evidence as to the relation of ovulation to the vaginal phenomena is presented in a later section.

Length of the cycle

The length of the cycle is most easily determined by counting between successive peaks of the curve, since bleeding was not an invariable accompaniment of the cycles. Counting in this way, we find for 991 six cycles of 23, (26), 17, 18, 16 and 20 days respectively. The long second cycle was due to an operation on the uterus which involved the removal of an endometrial sac thought to contain an implanted ovum. Omitting this cycle, the others average 18.8 days in length. For 992 we have recorded seven cycles, with lengths of 18, 16, 18, 18, 20, 17 and 19 days, an average of 18 days each. In the case of this second animal, bleeding was sufficiently regular to give five consecutive menstrual cycles; these were of 15, 20, 18, 20, and 16 days, for an average of 17.8. We may sum up by saying that in animals undergoing regular cycles the length of these cycles averages about 18 days, with a normal variability of 1 or 2 days greater or less than the mean.

Rengger's observations on *Cebus azarae* do not agree well with the above findings, either as to the length of the cycles or their regularity. Rengger says on this point (translation):

In the females I have observed now and then a kind of monthly flow, which however does not show any definite periodicity. It is quite scanty, lasts two to four days, and recurs sometimes after three, sometimes after six, and sometimes only after ten weeks. One sees this . . . first toward the end of the second year.

It is possible to understand these findings if we suppose that Rengger either was dealing with immature animals, in which the reproductive systems were going through the pubertal period of irregular functioning, or that he was not fortunate enough to have studied any females sufficiently healthy to show regular cycles. The latter explanation is probably the correct one; of the six adults I had under more or less regular observation, four were too irregular to give satisfactory data on the length of the cycles. This is merely another example of the only too well-known deleterious effect of captivity upon the reproductive functions of wild animals.

Menstruation

The finding in *Cebus* of a fairly regular, post-ovulatory bleeding during the low part of the vaginal cycle—hence undoubtedly menstrual in character—is of particular importance in view of the statements made by various authorities on Old World primates to the effect that menstruation does not occur in New World monkeys. Taken in connection with Goodman and Wislocki's findings in *Ateles* it points to the likelihood that menstrual phenomena may prove to be widely distributed throughout the *Cebidae*.³ It does seem, however, to be characteristic of *Cebus*, probably of the American monkeys generally, that menstrual bleeding is in lesser amount than in the macaque, baboon, or chimpanzee. No. 992 was quite regular in menstruating, only one of her observed cycles being amenorrheic, but only rarely was the amount of blood lost sufficient to appear externally, whereas in the catarrhine monkeys which have been studied bleeding is profuse enough for the menstrual period to be recognized without vaginal lavage. It is true that Rengger noted the bleeding, presumably without any special examination; but in my series, on the great majority of occasions, the blood was visible only in the lavages. The same scantiness of flow is reported in *Ateles*.

³ I have found bleeding also in *Chrysotrrix* and *Aotus*, but my data on these genera are not sufficient to show definitely that in these forms it is cyclic or menstrual.

Other individuals were less regular in bleeding than 992. Thus 991, although running consistent cycles of desquamation, bled only on the second, third, sixth, and seventh cycles. Of these, the first bleeding was due to a partial enucleation of the endometrium, while the last followed upon complete ovariectomy, consequently only two were spontaneous bleedings. Other females bled from one to four times during the course of the observations. Of six females observed for 6 weeks or longer, all menstruated at least once. In comparing these findings with those for Old World monkeys, it should be kept in mind that the latter also show variability in the regularity of menstruation. It is not infrequent in the macaque, for instance, to find cycles of desquamation unaccompanied by bleeding (Hartman, '32).

In every case, menstruation took place while the percentage of vaginal debris was in the low part of the cycle. In those animals in which the cycles were irregular or absent, bleeding occurred only once when the percentage was greater than 10. Bleeding may occur without any recognizable vaginal changes, as happened with immature female 2197, and in no. 3126 who was nursing a baby when caught.

Although the bleeding appeared scantier than in other primates, the duration seems to be about the same in all. The duration of the menstrual flow in 992, for the various periods, was as follows: 2, 5, 4, 5, 3 and 7 days, an average of 4.3 days. In other females it lasted from 1 to 5 days; usually 1 or 2 days with the blood plainly visible in the lavage, then 1 or 2 days with the washings only faintly tinged or with a few red blood cells detectable only under the microscope.

Cytology of the lavage

Microscopic examination of the vaginal lavages revealed the presence, together or at succeeding times, of the usual nucleated epithelial and cornified squamous cells, leucocytes, and red corpuscles. The relative numbers of these varied in a definite and significant fashion. While the details of these changes were worked out mainly from the two animals of

figure 1, they were also found in other animals whenever these exhibited a definite cycle in the amount of sediment.

At the onset of menstruation, the vaginal sediment consisted mainly of relatively small, nucleated epithelial cells. There were also a few squamous cells and leucocytes, and of course many erythrocytes. The number of these latter, however, usually seemed inadequate to color the lavage as brightly red as it was at first; it seems probable that many of the corpuscles were hemolyzed before reaching the vagina and that much of the color was due to hemoglobin in solution in the cervical exudate. Granular debris and mucus were occasionally present at this time.

The close of menstruation was marked only by the disappearance of the erythrocytes. During the early part of the postmenstrual phase the epithelial cells continued to be the dominant element in the lavage, and this epithelial phase is characteristic of animals not exhibiting cyclic changes. As soon as the amount of sediment began to increase, however, the squamous cells multiplied rapidly in number and prominence; in fact, the rise in the curve is directly due to the increasing number of cornified cells shed from the vaginal wall. Sometimes the increase of cornified cells began before menstruation was completed, in these cases the curve started to rise during the menstrual period. Irrespective of when it began, the ascending phase of the curve was characterized by the ever increasing number of cornified cells in the vaginal debris. At the peak, when the sediment formed 40, 50 or 60% of the lavage, this detritus was an almost pure mass of squamous cells. Ovulation probably occurred at this time.

Immediately following the peak of vaginal activity there was a sudden and dramatic change in the cellular composition of the lavage. One day the sediment would stand at 50 or 60%, containing practically nothing but cornified cells which gave it a white, curdled-milk appearance. The day following, the lavage would be cream colored, the percentage of sediment 10 or 20 points less, while the microscope would reveal that now its volume was at least half leucocytes. This sudden

shower of leucocytes was a constant accompaniment of the drop from the peak of the desquamation curve. It was never of long duration, after 2 or possibly 3 days the leucocytes disappeared together with most of the cornified cells. The epithelial cells now became once more the dominant element of the picture, and by the time menstruation recurred squamous cells are practically absent.

To sum up briefly the foregoing account: nucleated epithelial cells formed the greater part of the vaginal sediment before and during menstruation, when the lavage was relatively free from debris. The increase in the percentage of sediment following menstruation was due to an increasing exfoliation of cornified cells, a desquamation which reached its climax at the peak of the sediment curve and then fell again within 2 or 3 days to its low level. Immediately following this maximum desquamation, a flood of leucocytes temporarily invaded the vagina, only to vanish again within 72 hours. With the practically complete disappearance from the lavage of squamous cells and leucocytes, the percentage of sediment returned to the low level characteristic of the menstrual period.

Time of ovulation

From the very beginning of the study, a comparison of the vaginal cycle with that of the spider monkey and macaque indicated that ovulation probably took place during the high part of the curve. This was confirmed during three laparotomies, two on no. 991 and one on 992, performed to try to recover fertilized ova. These operations were done 6, 8 and 12 days respectively following the peak of the sedimentation curve. In each case a fresh corpus luteum was found, of an apparent age corresponding to the length of time elapsed since the peak. It must be borne in mind that it is not possible to determine the age of the corpus luteum with extreme exactitude, so that it was impossible to say whether the follicle which gave rise to the corpus had ruptured at the peak of the curve, or shortly before, or shortly afterward. In order

to determine more exactly the moment of ovulation, a double laparotomy was done on female 991 in the following circumstances.

Monkey 991 showed a lavage, on the morning of April 15th, consisting of squamous cells, non-motile spermatozoa, and seminal coagulum, indicating that copulation had taken place during the night. This was the seventh day of her menstrual cycle, 17 days following the previous peak, and the vaginal curve was well along in the ascending postmenstrual phase. The following day, April 16th, there was 35% of sediment, the washing being a practically pure suspension of squamous cells. Laparotomy showed an enormous (7 to 8 mm.) follicle in the right ovary. The left ovary contained three old corpora lutea of various ages, it was removed for preservation. Vaginal sediment the following day was 40%, all squamous cells; the next day it was 55% and still consisted exclusively of cornified cells. The following morning, April 19th, the sediment had dropped to 22%, and was now a mixture of cornified cells and leucocytes in approximately equal volumes. The animal was at once opened and the right ovary removed. The large follicle seen in it 3 days previously had discharged, but so recently that it did not as yet show any formation of luteal tissue. Its age was estimated as probably less than 24 hours, and it was certainly younger than 2 days.

The foregoing observations place the moment of ovulation within a few hours of the climax of vaginal desquamation. It must be remembered that only one lavage was taken daily; hence the time when the desquamation reached its maximum, also the first appearance of leucocytes in numbers, can only be determined to the day. From the estimated age of the ruptured follicle (incipient corpus luteum) at laparotomy, we may say that ovulation seems to have occurred on the same day as the maximum of vaginal desquamation, and on the day preceding the leucocytic invasion of the vaginal lumen. It will not be possible to be more precise until the observations can be repeated with the vaginal lavage taken several times daily and with a daily laparotomy during the three or four critical days.

Adolescence and pregnancy

Observations on young and on pregnant animals may be compared briefly with those for normal adults. Female 2197 was alleged to be an adult when received, but after several weeks of lavages had failed to yield more than a trace of sediment at any time suspicions were aroused as to her actual age. Examination showed that she possessed first and second molars, with the third lower ones just beginning to erupt. This would indicate an age of 3 years or more, according to Rengger's records of tooth eruption in *Cebus azarae*, an age which agrees with my own estimate based on comparison with various young monkeys of diverse ages. Although 2197 was not showing any vaginal cycles, nevertheless she bled four times at irregular intervals while under observation; on the twenty-ninth day following the start of the experiment, then at intervals of 8, 35 and 37 days. The bleeding was very slight, twice lasting but a single day and twice 3 days. This irregular and slight bleeding, unaccompanied by definite vaginal cycles, is probably characteristic of adolescent animals; it agrees with Hartman's observations on young macaques. In my opinion, the animal cannot have been menstruating long, and I would consequently place the onset of this phenomenon toward the close of the third year, considerably later than stated by Rengger. It would require probably another year for full reproductive function to be established, which means that this species of *Cebus* would not breed before the age of 4 years.

Two animals were examined in the latter half of pregnancy. Both yielded practically sediment-free lavages. One showed a few epithelial cells, many being of abnormal forms; the other had a few epithelial cells and a few erythrocytes. Female 3126 was suckling a baby when captured, she likewise showed an almost clear lavage at the time of capture and for several weeks after being separated from the baby. Here again we find a close similarity to the conditions in pregnant and in lactating rhesus monkeys.

Comparison with other animals

The form and cytology of the vaginal curve show interesting comparisons with those of certain other animals. The steepness of the curve, in both pre-ovulatory and post-ovulatory phases, is markedly greater than that of the macaque; furthermore, the amount of desquamation usually attains a somewhat higher maximum in *Cebus*. Again, in its single-peaked curve our species is unlike the rhesus monkey, in which there are frequently two maxima of the vaginal exfoliation in a single intermenstrual interval; in this respect *Cebus* and *Ateles* agree with each other and differ from *Macaca*. On the other hand, there is but a single peak in the curve of perineal swelling in the baboon; this of course may be quite unlike the sequence of events in the vagina. A feature in which *Cebus* differs from all the other primates studied is in the length of the cycle; 18 days as against 28 to 35 or 42 in the others.

Insofar as the cellular changes are concerned, *Cebus* seems to be definitely simpler than the catarrhine monkeys, apes, and human. The massive exfoliation of cornified cells at the time of ovulation, followed within 24 hours by a flood of leucocytes, is startlingly similar to what occurs in the rat. The picture is complicated in the higher primates by variations in the numbers of the epithelial cells, and by more than one leucocytic invasion. There seems to be a tendency in all forms for cornified cells to appear around the time of ovulation; this has been noted in macaque, baboon, chimpanzee, and woman. The lavage in these others, however, is seldom such a pure suspension of squamous cells as it is in *Cebus*, but contains an appreciable number of epithelial cells and, especially, leucocytes; nor do the squamous cells disappear so suddenly as they do in *Cebus* following the appearance of the leucocytes. The cornified cell stage is apparently common to all mammals; its better demarcation and emphasized importance in *Cebus* as compared to the catarrhines may be evidence of a more primitive reproductive physiology in the American monkey.

We may conclude that the vaginal cycle of *Cebus* is definitely simpler (and more primitive?) than in the higher primates. It shows marked resemblances to the rat in the cellular changes in the vagina, but is typically primate in the presence of a menstrual bleeding which follows several days after infertile ovulation.

ESTROUS CYCLE AND SEX BEHAVIOR

Contrary to the habit of the Old World monkeys, *Cebus* appears to copulate only at a definite time in the cycle. A vaginal plug and spermatozoa were found ten times in thirteen cycles in females 991 and 992. In every case copulation took place either at the peak of the vaginal cycle, or during the preovulatory rise toward the peak, but never more than 3 days prior to it. This would indicate that copulation is restricted to the time of ovulation, or just before it. Copulation was never found with three other females, confined in the same cage, in which cycles were irregular and poorly marked, and which were probably not ovulating.

The association between sexual urge and the time of the cycle thus appears to be much stronger in *Cebus* than in the macaque or the chimpanzee. Ball and Hartman ('35) for the macaque, Elder and Yerkes ('36) for the ape, have shown that copulation occurs most frequently during the ovulatory period; nevertheless, in both ape and macaque, pairing does take place, though less freely, at all other times of the cycle. Judged on the basis of the present limited observations, *Cebus* is apparently somewhat more primitive in this respect; that is, it is more like the lower mammals which pair only at estrous, and less like the human.

It is worthy of note that copulation was never actually observed, although the monkey cage was in full view from the laboratory windows and was under fairly close observation by the staff and assistants from 7.00 A.M. until 5.00 or 6.00 P.M., and although the assistants had been given orders to watch for and report any sexual activities. It seems unlikely that the monkeys copulate at night; more likely pairing took place at daybreak, before the arrival of the attendants.

At all events the sexual behavior of the monkeys under observation was quite different from the uninhibited and frequently exhibitionistic behavior of rhesus monkeys under similar conditions.

BREEDING SEASON

The observations on the cycles in captive monkeys, and on pregnant or nursing monkeys caught in the forest, indicate that *Cebus* in Southern Goyaz has no limited and well-defined breeding season but may breed and bear young at any time of the year. Evidence for this is presented briefly.

The first observations were begun early in October. These were interrupted in November by the necessity of arranging larger quarters for the animals; nevertheless they showed definitely that the females showed normal vaginal cycles at that time. Monkey 991 was operated on October 24th, following a well-marked cycle; the left ovary contained a fresh corpus luteum, the right ovary showed two older corpora, thus proving that the cycles at that time of the year were functional and not anovulatory cycles such as occur in the macaque during the non-fertile summer season.

With the resumption of the experiments, the animals were under continuous observation from early December until the end of the following April, two of them showing regular cycles all this time. Female 991 was laparotomized again in January and in April, no. 992 was ovario-hysterectomized in April. On each occasion the ovaries contained several corpora of varying ages, from very fresh to quite old, leaving no doubt but what the great majority, probably all, of the cycles had been ovulatory ones. We thus have evidence from our captive animals that ovulatory cycles continued from October through April—7 months of the year.

Female 3126 was released, non-pregnant, in June 1937, and was recaptured in December of the same year with a young baby. Pregnancy thus lasted less than 6 months, and the animal had bred successfully in June or later. This is normal for the species, for records on monkeys captured in the forest show October and November as a time when many births

occur. We can accordingly extend the period in which ovulatory cycles occur to include June or July, which makes it cover 9 or 10 months of the year.

It seems certain, therefore, that the non-breeding season in *Cebus* cannot be of more than 2 or 3 months' duration, if in fact it exists at all. It is unfortunate that we have no information for August and September, but I venture to predict that the cycles will eventually be shown to continue through these months also, giving *Cebus* a continuous, year long breeding season interrupted only by pregnancy and lactation or by disease. This definitely does not occur in all South American monkeys, as I shall show in another paper; nor does it mean that in *Cebus* births occur with uniform frequency at all times of the year. As a matter of fact, the dates of capture of baby monkeys and of pregnant females indicates that the majority of births, in Goyaz, are divided between May-June and October-November. Fertile matings must therefore be more frequent at two different times of the year, even though the non-pregnant female runs cycles continuously. This would be easy to understand if there were a non-fertile period of anovulatory cycles, such as is found in the macaque, but all the evidence from laparotomies goes to show that anovulatory cycles are the exception and not the rule in *Cebus*. The reason for the bimodal seasonal curve of conceptions in *Cebus* must remain a mystery for the present.

SUMMARY

Captive individuals of *Cebus ?azarae*, in Southern Goyaz, showed the following characteristics of the reproductive cycle and sexual behavior:

1. There was, in healthy females, a regular estrous cycle detectable by vaginal lavage.
2. The length of the cycle was 16 to 20 days, with an average of 18 days.
3. Ovulation occurred at, or close to, the time of maximum vaginal desquamation. Anovulatory cycles were rare or absent in animals showing regular vaginal changes.
4. Menstrual bleeding took place in many of the cycles, in some females in practically all cycles.

5. Menstruation, when found, occurred when vaginal desquamation was at its lowest. The bleeding lasted 1 to 5 days, and was rather scanty.

6. The preovulatory part of the cycle was characterized by increasing vaginal exfoliation of cornified cells. Following ovulation there was a sudden, massive invasion of the vaginal lumen by leucocytes.

7. An adolescent animal showed practically no vaginal sediment and menstruated at infrequent, irregular intervals. Pregnant females and lactating females likewise showed little or no sediment in the vagina.

8. Copulation took place only at, or shortly prior to, the time of ovulation. A vaginal plug was formed by coagulation of the ejaculate.

9. Ovulatory cycles ran through at least 9 months of the year. There are at present no observations from July through September. Births of young monkeys, and hence fertile matings, occur most frequently at two times of the year. The reason for this bimodal curve of conceptions, when ovulatory cycles are apparently continuous, is not evident.

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AN ANOMALOUS CERVICO-OCCIPITAL SKELETON IN MAN

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THREE PLATES (TWELVE FIGURES)

An anomalous cervico-occipital junction was observed in the vertebral column of a white male 54 years old, the striking feature being a structure resembling a complete atlas fused to the base of the occipital bone. Hardly less remarkable is the epistropheus which is formed by elements of two vertebrae fused cranio-caudally on the right side and presenting on the left a separate ossicle similar to the fused cranial element. The next three cervical vertebrae are normal in all details. The bodies of the last two vertebrae exhibit lipping on their adjacent anterior margins. The last cervical vertebra, usually the seventh, has greatly elongated transverse processes whose costal elements approach the definitive shape of ribs. Except for the mentioned exostoses there is no apparent pathology in the cervical skeleton, nor does radiology reveal any pathological fusion. It is interesting that structures of eight vertebrae compose the cervical part of the vertebral column. The thoracic region has eleven normal rib-bearing vertebrae, there is one thoraco-lumbar vertebra, and five lumbar vertebrae. The sacrum is composed of five vertebral elements with a fused part of the coccyx. The remainder of the coccyx is a free, indeterminate nodule.

A well-formed atlantal structure is fused to the base of the skull around the rim of the foramen magnum by its anterior arch, lateral masses, and posterior arch. It is more closely drawn into the occipital bone on the left than on the right, and it is rotated to the right approximately 10° .

The anterior arch is prominent, and joined extensively from side to side to the basilar part of the occipital bone. On the right of the mid-line externally, there is a deep, smooth-walled groove between the anterior arch and the basilar part of the occipital which extends in a lateral direction 6 mm. This right anterior atlanto-occipital groove narrows in the depth, and communicates with the anterior space of the vertebral foramen by a slight fissure between the anterior arch and the pars basilaris. On the left side there is a large rough-walled groove in a transverse plane between the external surface of the pars lateralis of the occipital bone and the site of the condyle and lateral mass of the atlantal vertebra. This left anterior atlanto-occipital groove extends from a point 7 mm. left of the mid-line, laterally to the level of the incomplete transverse foramen of the atlantal element, and reveals the limits of the lateral mass toward the skull. Into this deep groove open the left hypoglossal canal, incompletely bi-partite, and a canal which probably transmitted a persistent hypoglossal artery (Keibel and Mall). Whereas the right anterior atlanto-occipital groove separates both the basilar and lateral parts of the occipital bone from the anterior arch of the atlantal vertebra, the left groove is limited almost entirely to the space between the pars lateralis and atlantal lateral mass. The anterior tubercle of the anterior arch is medium in size.

The facet on the posterior surface of the anterior arch of the atlantal element for articulation with the odontoid process is large, oval, with slight projections of the surface superiorly and inferiorly. Its center is slightly to the right of the midline. The left margin of the facet is free and overhangs the inner surface of the lateral mass. This projection gives the facet a marked transverse concavity.

The lateral masses are strongly fused to the base of the skull; rough grooves, more strongly marked on the left than on the right, may show the lines of fusion internally. The posterior extent of each anterior atlanto-occipital groove is opposite the line of fusion externally. Each lateral mass

presents an inferior articular facet. The inferior articular facet on the left is elongate and irregularly concave. Its long axis extends anteriorly and medially and it is prolonged upon the anterior arch quite to the facet for the odontoid process. Here the surface is inclined slightly anteriorly and laterally as well as downward; the posterior lateral end of this articular surface is inclined downward, slightly anteriorly and medially. The right inferior articular facet, oval, nearly flat, has the position and inclination of the normal inferior articular surface of an atlas. The horizontal levels of the two inferior articular surfaces are not the same, the right reaching a considerably lower plane. Tubercles for the attachment of the transverse atlantal ligament are obscure, if present. The form of the vertebral foramen is unsymmetrical, resulting from the forward displacement of the left inferior articular process on the anterior arch.

The right transverse process of the atlantal vertebra is separated from the base of the skull by 8 mm. distance. It has no complete transverse foramen, the costal element being represented by a slight spicule of bone extending from the lateral mass approximately 3 mm. The strong transverse element ends free in a blunt tubercle in close proximity to the tip of the mastoid process. The jugular process of the occipital bone on the right sends inferiorly a projection as a paramastoid process located medial to the mastoid process and anterior to the level of the transverse element of the right atlantal transverse process. It ends bluntly on a plane with the spicule representing the costal element of this transverse process. The left transverse process of the atlantal vertebra is short and irregular. It lies on and partly fused with the pars lateralis of the occipital bone; a fissure separates the root of this transverse process from the pars lateralis, and extends anteriorly to open into the left anterior atlanto-occipital groove. A small projection from the lateral mass toward the anterior lateral edge of the left transverse process suggests a rudimentary costal element. The posterior medial wall of the jugular foramen is fused with the

anterior edge of the left transverse process, and projects medially toward the rudimentary costal element. The transverse foramen on the left, therefore, is incompletely bounded by costal and transverse elements of the transverse process, and by the posterior medial wall of the jugular foramen.

The posterior arch of the atlantal element is complete. It is incompletely united by suture to the posterior rim of the foramen magnum near the mid-line for approximately 1 cm. From this region nearly to the left lateral mass the posterior arch, thin and compressed internally-externally, is separated from the occipital squama by a narrow irregular fissure. Here the superior edge of the posterior arch is sharp and rough. There is no groove for the vertebral artery. To the right of the mid-plane the posterior arch is solidly fused with the squama next to the foramen magnum for a distance of 7 mm., but is free toward the right lateral mass. This right half of the arch is broad and compressed supero-inferiorly near the lateral mass; and is narrow and prismatic medially; its plane is close to the base of the skull. A well-marked groove for the right vertebral artery is present upon the superior surface of the posterior arch, posterior to the lateral mass.

It has been mentioned that there is no groove for the vertebral artery on the superior surface of the posterior atlantal arch on the left. The inferior surface of the posterior arch on the left is grooved posterior to the lateral mass, indicating, we believe, the passage of the vertebral artery here between it and the rudimentary posterior arch on the separate ossicle to be described. There is, however, a canal leading from the anterior atlanto-occipital groove above the root of the left transverse process to open in the left inner margin of the foramen magnum. This canal, we believe, transmitted the persistent hypoglossal artery already mentioned.

The rim of the foramen magnum superior to the posterior atlantal arch on the left, is prominent and partially separated externally from the squama occipitalis by a conspicuous groove. Shallow at its beginning on the squama approximately 6 mm. to the left of the mid-plane posteriorly, this

groove deepens progressively as it courses forward parallel to the margin of the foramen magnum, and terminates by forming a canal extending superiorly, forward, and medially, to open into the canal described for conducting, possibly, the hypoglossal artery at the inner limits of the latter on the rim of the foramen magnum. The ridge of bone thus formed between the groove and the space separating the posterior arch of the atlantal vertebra from the occipital squama is a strong labium foraminis magni, increasing in size from behind forward, and which is continuous anteriorly and laterally with the lateral mass and root of the transverse process of the atlantal vertebra. The labium is separated from the squama occipitalis internally by a rough-edged, narrow fissure beginning in a faint line anteriorly toward the superior edge of the inner opening of the canal for the hypoglossal artery, and extending to a point on the margin of the foramen magnum near the mid-line posteriorly. There is no labium posteriorly on the right. Indications of anterior labia of the foramen magnum are doubtful. An internal ridge extending from side to side superior to the continuous groove separating the atlantal lateral masses and anterior arch from the pars basilaris and lateralis of the occipital bone, marks the anterior margin of the foramen magnum.

The epistropheus is asymmetrical. On the right side there appears to be fusion of two or more half vertebrae. The accretion of the right extends beyond the mid-line to include anteriorly the odontoid, and posteriorly the lamina; the remainder of the vertebra is fairly normal but presents certain anomalies, to be mentioned later. The odontoid process is irregular in form, and more pointed than usual; the tip bends to the right and posteriorly; its facet for the anterior arch of the atlas likewise is inclined posteriorly and to the right. An articular surface for the transverse atlantal ligament is present. Upon the left side of the base of the odontoid process is an elongated articular surface extending up and down, facing laterally and slightly anteriorly. Near its posterior limit is a (vascular ?) foramen. This surface articu-

lates with the medial extremity of the free ossicle of the left side of the composite epistropheus as described below.

The anterior articular facet of the epistropheus on the left approaches the normal in shape and position; it is, however, concave meso-laterally instead of convex, and somewhat smaller than usual in area. It does not project laterally, partially to roof the transverse foramen as in a normal epistropheus, and the transverse foramen is in consequence, directed superiorly instead of supero-laterally. This facet articulates with a separate bone, to be described later, that intervenes between the said facet of the epistropheus and the vertebra fused to the occipital bone. Its medial edge is 21 mm. inferior to the level of the tip of the odontoid process. The left transverse process, the left lamina of the neural arch and its inferior articular facet are not unusual in position and development. The anterior articular facet on the right is oval in outline, larger, and at a higher plane than the facet on the left. The medial edge of the right facet is 13 mm. inferior to the level of the tip of the odontoid process, and 8 mm. superior to the level of the medial edge of the left facet. The right facet slopes laterally and posteriorly; it is concave meso-laterally and slightly so anteroposteriorly. The right anterior facet surmounts the bony mass which, as will be seen from the description following, can be regarded as developed from the neural, costal, and chordal elements of a composite vertebra. This element, present on the right side only, is continuous medially with the odontoid process, inferiorly with the body, and posteriorly with the lamina of the composite epistropheus. The element has a transverse process with a complete transverse foramen, the costal and transverse elements being short and ending bluntly in tubercles. As on the left, this transverse foramen is not overhung by the anterior articular facet. Between the roots of the upper and lower epistropheal elements on the right there is a complete intervertebral foramen. Faint lines mark the fusion of the lamina and body of the upper half vertebra with the lamina and body of the lower vertebra. The lines of separation between the two laminae indicate that the lamina of the upper

vertebra is not associated in making the massiveness of the spinous process. This upper lamina is broad next to the root, but tapers rapidly almost to a point near the dorsal mid-line.

The spinous process is large, roughened, and trifold, two of the three processes being on the right of the mid-plane. The superior one of the two on the right is approximately symmetrical to the single process on the left. The inferior process on the right is longer than the others. It is hooked, like a beak, posteriorly and inferiorly. The remainder of the inferior epistropheal vertebra on the right is not exceptional.

The separate ossicle is on the left and occupies a position between the inferior facet of the fused atlantal mass above and the anterior facet of the inferior epistropheal element below. Except for the odontoid process it is comparable in form to the fused superior half-vertebra on the right side of the epistropheal vertebra. It consists of a wedge-shaped lateral mass with a base laterally and an apex medially. It has superior and inferior articular facets, a transverse process with a foramen, and a rudimentary posterior arch. The superior facet is adapted in its contour for articulation with the irregular inferior facet of the atlantal vertebra fused to the occipital bone. The inferior articular facet of the ossicle resembles that of a normal atlas. It articulates with the anterior facet of the epistropheal vertebra on the left. The blunt apex of the little bone is somewhat compressed antero-posteriorly, and presents an articular surface which comes into contact with that at the antero-lateral side of the base of the odontoid process when the ossicle is in place. In the formation of the transverse foramen the costal element is larger than the transverse. The two elements end in a single tubercle. The rudimentary posterior arch is 8 mm. long. It is flattened from above downward and curves from the lateral mass posteriorly and medially. The upper surface of the rudimentary posterior arch presents a transverse groove suggesting the groove for the vertebral artery on the arch of a normal atlas posterior to the lateral mass. The level of the superior articular facet of the ossicle is approximately

the same as the anterior articular facet on the right side of the epistropheal vertebra when the ossicle is in place.

The following four cervical vertebrae are normal. The last of the cervical vertebrae, usually the seventh, has abnormally long transverse processes which have a resemblance to those in the thoracic region. The left transverse foramen of the last cervical vertebra is elongated laterally. The costal process expands into a head where it joins the body of the vertebra; laterally it is fused to the transverse process. The tip of the left costal element is damaged, but all indications point to its extension beyond the transverse element of the process. In the line of fusion of the costal and transverse elements on the left there is an irregular foramen. On the right the costal element of the transverse process has been broken close to the body of the vertebra and again just before meeting the transverse process. On this side, as on the left, there is an irregular foramen in the line of fusion between the costal element and the anterior inferior margin of the tip of the transverse element. The costal element on the right extends beyond the transverse element and curves inferiorly, laterally, and forward. It extends 8 mm. beyond the transverse element, ending in a blunt point.

In summary: an atlantal vertebra is fused with the skull, on the left more strongly than on the right. It is twisted to the right about a central vertical axis. The epistropheal vertebra appears to have a half-vertebra fused superiorly to its right side. The half-vertebra adds to the odontoid process, lateral mass and its processes, and the right lamina of the posterior arch. The inferior aspect of the epistropheal vertebra is normal. Its spinous process resembles the close fusion of two spinous processes, both bifid, with fusion more complete on the left. There is a free ossicle comparable to the fused mass of the right of the epistropheus. The transverse processes of the last cervical vertebra are elongated; their costal elements suggest the shape of little ribs.

COMMENT

Certain peculiarities in the *pars basilaris* of the occipital bone and the superior part of the cervical segment are obvious after a study of the specimen described. There is the absence of the groove for the vertebral artery on the left side of the posterior arch of the atlantal vertebra posterior to the lateral mass of the first vertebra. The greatest fusion of the atlantal vertebra involves its left lateral mass and the posterior arch immediately adjoining. The inferior articular facet of the atlantal vertebra on the left deviates in shape and inclination from that of a normal atlas and from the inferior facet on the right. There is an extra foramen described, probably for a hypoglossal artery. There is the marked asymmetry produced in the epistropheus by its fused addition, and finally, there is an apparent 'intercalation' into the series of cervical elements.

It is unlikely that intercalation of a new element into the series of vertebrae ever occurs. Addition to one region of the vertebral column usually is accomplished by shortening of another. It is possible to explain the addition to the described cervical region of a column whose other vertebral segments have the prescribed number of elements, by assuming the partial 'manifestation of an occipital vertebra' concurrently with 'assimilation of the atlas.'

Swjetschnikow ('06) and Bystrow ('31) list certain characteristics of the two phenomena, 'manifestation' and 'assimilation.' Among the characteristics of atlas assimilation are: 1) a foramen or canal between the anterior arch and the occipital, called the atlanto-occipital space; 2) frequent development of the paramastoid processes; 3) presence of a canal for the vertebral artery and the first cervical nerve located between the posterior arch of the atlas and the base of the occipital bone; 4) retention of the normal shape and inclination of the inferior articular facets of the atlas.

The present specimen shows great asymmetry on the right and left sides of the atlas in respect to these four points. On the right all four points indicate assimilation of the atlas; on

the left all four points indicate manifestation of an occipital vertebra. Moreover, if the separate ossicle is articulated with the left inferior facet on the base of the occipital region it is seen to lie in the same plane with that part of the element fused with the occipital whose right lateral mass, anterior arch, and posterior arch are in agreement with criteria for an assimilated atlas. The separate ossicle resembles the lateral mass of an atlas. If it is substituted for that element on the left of the foramen magnum on the base of the skull which answers criteria for manifestation of an occipital vertebra, it very nearly completes a 'normal' atlas.

In continuing to apply this explanation to the formation of the anomalous cervical region here described, certain well known observations are briefly mentioned. The phenomenon known as 'manifestation of an occipital vertebra' is attributed to incomplete incorporation of the last occipital sclerotome into the base of the occipital region (Kollmann, '07, and others). The comparative anatomical studies of Lachi, and Chiarugi (from Swjetschnikow, '06) and the embryological investigations of Terry ('17) and Weiss ('01) have shown that the bodies of the atlas and of the occipital vertebra are similarly separated from the rest of their elements. The epistropheus takes up the normal mode of development for the vertebral series, but has added to it the blastemal body of the atlas which Frioriep (1886) and Macalister (1894) found to produce the odontoid process and the anterior articular processes of the epistropheus. The fusion on the described epistropheus can possibly be explained as the result of addition to the blastemal body of the usual atlas a blastemal body resulting from the partial manifestation of an occipital vertebra.

The manifestation of the occipital vertebra would have produced a crowding of the sclerotomes on the left by pushing in from above, whereas the fusion of the atlas on the right would increase the space between the occipital sclerotome and those of the axis on that side. Irregular segmentation must have occurred in the sclerotomes on the right, posterior

to the anlage of the atlas, to produce the duplicate epistropheal mass fused on the superior surface of the second vertebra. This opinion follows the suggestion by Danforth ('30) and Bystrow ('31) that the form of a vertebra depends on its position in the segmental series.

According to Haffner ('36) these variations would have been established before the end of the fourth week of development. Haffner finds reason to support the earlier ideas of Müller, Frey and Stiebe (Haffner, '36) that the blastemal halves of vertebral anlagen are independent in segmentation and differentiation through the fourth week. Werenskiöld ('37) adopts this theory for an explanation of anomalous conditions present in a spine quite homologous to the one described here.

Both Haffner and Werenskiöld look to genetics for the fundamental etiology of their described anomalies. Kühne ('36) who has established the inheritance of either cranial or caudal shifts at the cervico-thoracic, thoraco-lumbar, lumbo-sacral, and sacro-coccygeal borders, states that manifestation of an occipital vertebra is a cranial shift, and that assimilation of the atlas is a caudal shift at the occipito-cervical junction, but that he is inclined to suspect no correlation between occipito-cervical shifts and shifts at other vertebral borders. None of his specimens presented atlanto-occipital variations, whereas the two cases he selected from Hasebe's series showing assimilation of the atlas were variant, one being a cranial and the other a caudal type column. Kühne did note that among the Japanese, caudal shifting columns are more numerous than in Europeans, and that atlas assimilation is also more frequent among the Japanese.

The vertebral column here described is one with strong cranial shifts at the cervico-thoracic and thoraco-lumbar borders. The last cervical vertebra is described, having thoracic characters. Vertebra 19 is thoraco-lumbar, with superior articular processes of the lumbar type; it is without rib or transverse process on the right. The lumbo-sacral

border is normal, and the sacro-coccygeal is regarded as normal in spite of the fusion of the part of the coccyx, which appears to be an age change.

The atlanto-occipital border shows all the morphology of a cranial shift on the left and a caudal shift on the right. This alone indicates independence of the border from the influences dominating the other intersegmental regions. It would be desirable to check this observation on as large a series of specimens as possible.

For this purpose the writer examined the pars occipitalis of 1246 skulls in the Washington University Collection. Of these, thirteen skulls (1.04%) exhibited two or more characters of manifestation of occipital vertebra (Kollmann and Swjetschnikow). There were ten of these specimens with definite cranially varying vertebral columns; the other three are vertebral columns which are neither cranial nor caudal types. The first and last pairs of ribs were available for comparison in one of the three neutral columns, and the twelfth ribs were found approximately 1 cm. longer than the first. Adolphi ('05) correlates this with columns varying caudally. Of the columns, therefore, ten indicate cranial shifting, two are neutral, and one may have slight caudal shifting tendencies.

Assimilation of the atlas is shown by two skulls (0.16%), and the vertebral columns of both these specimens are unmistakably cranial shifting types. The described specimen is not included in any of the above figures.

SUMMARY

1. A specimen is described exhibiting concomitant assimilation of the atlas and manifestation of an occipital vertebra with correlated modifications in the cervical region of a vertebral column whose other intersegmental borders shift cranially.

2. Variation at the cranio-vertebral border in a small series seems unassociated with variations at the lower intersegmental vertebral borders, and therefore is unassociated with the hereditary control of the latter as established by Kühne.

I wish to thank Dr. R. J. Terry for his guidance throughout the preparation of this paper.

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PLATE I

EXPLANATION OF FIGURES

- 1 Inferior view of the base of the cranium. a, jugular fossa; b, facet on the anterior arch of the atlantal vertebra for the dens epistrophei; c, inferior articular facet of the atlantal vertebra; d, tubercle on the posterior arch of the atlantal vertebra; e, transverse element of the atlantal transverse process; f, paramastoid process.
- 2 Posterior view of the base of the cranium. a, facet on the anterior arch of the atlantal vertebra for the dens epistrophei; b, inferior articular facet of the atlantal vertebra; c, transverse element of the atlantal transverse process; d, canal for the right vertebral artery; e, groove for the left vertebral artery; f, posterior labium of the foramen magnum.
- 3 Medial view of the right half of the cranium. a, anterior arch of the atlantal vertebra; b, posterior arch of the atlantal vertebra; c, canal for the vertebral artery; d, inferior articular facet of the atlantal vertebra; e, hypoglossal canal.
- 4 Medial view of the left half of the cranium. a, anterior arch of the atlantal vertebra; b, posterior arch of the atlantal vertebra; c, groove for the vertebral artery; d, internal aperture of the canal for the hypoglossal (?) artery; e, hypoglossal canal.

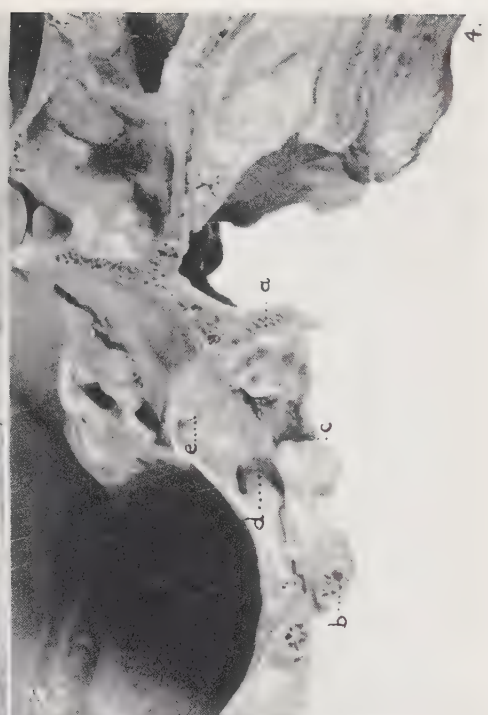
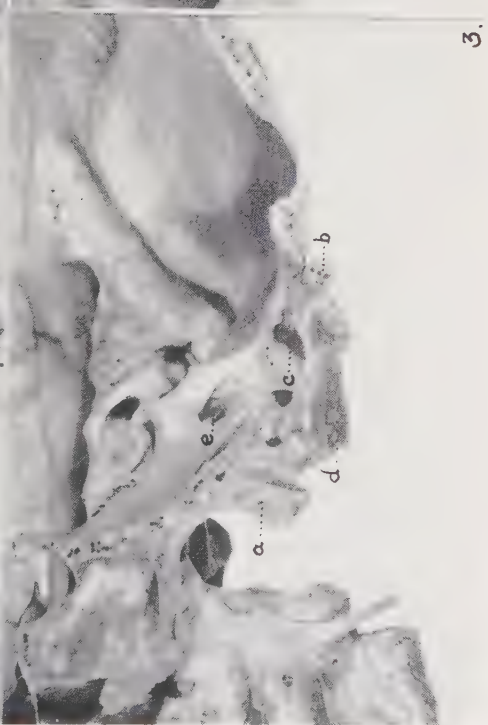
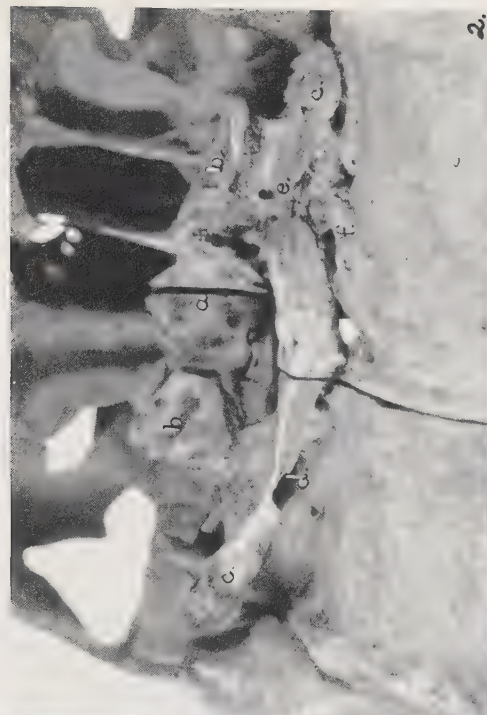
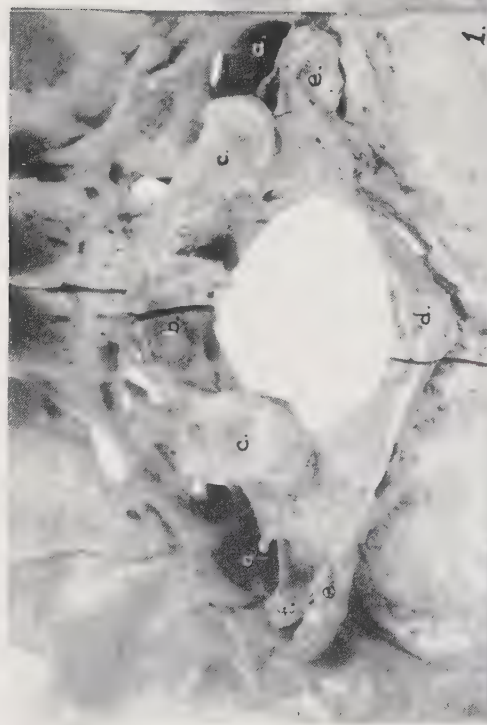


PLATE 2

EXPLANATION OF FIGURES

5 Anterior view of the epistropheal vertebra. a, facet on the dens for articulation with the atlantal anterior arch; b, facet on the dens for articulation with the apex of the ossicle; c, position of the anterior articular facets; d, lateral mass of the element fused with the epistropheus.

6 Right lateral view of the epistropheal vertebra. a, dens epistrophei; b, anterior articular facet of the element fused with the epistropheus; c, transverse process of the element fused with the epistropheus; d, 'intervertebral' foramen between the epistropheus and the fused element; e, lamina of the element fused with the epistropheus.

7 Superior view of the separate ossicle. a, superior articular facet; b, transverse process; c, groove on the rudimentary posterior arch for the vertebral artery.

8 Inferior view of the separate ossicle. a, inferior articular facet; b, transverse process; c, rudimentary posterior arch.

9 Lateral view of the separate ossicle. a, superior articular surface; b, transverse process; c, rudimentary posterior arch; d, inferior articular surface.

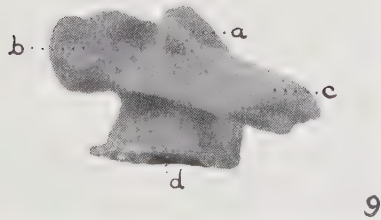
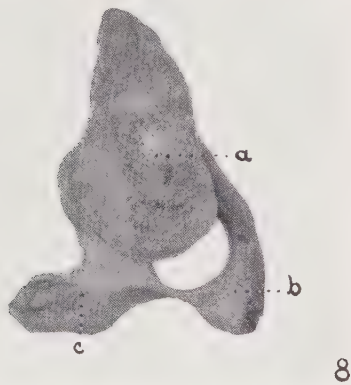
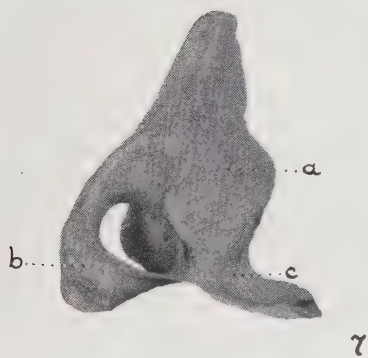
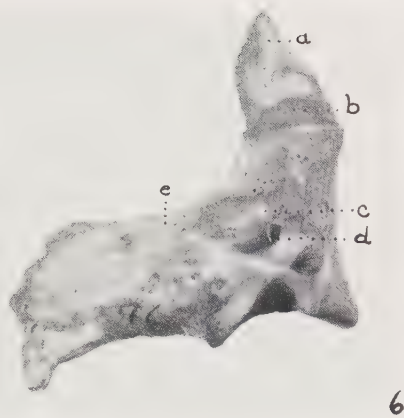
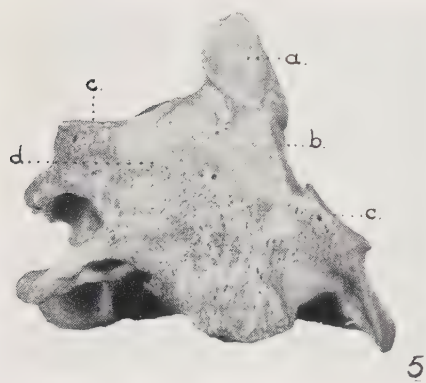


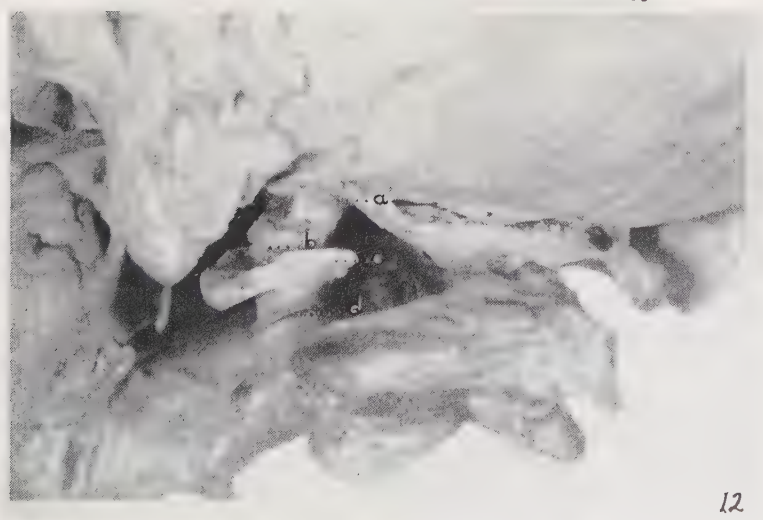
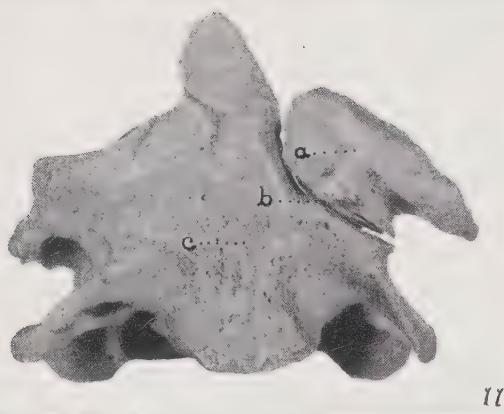
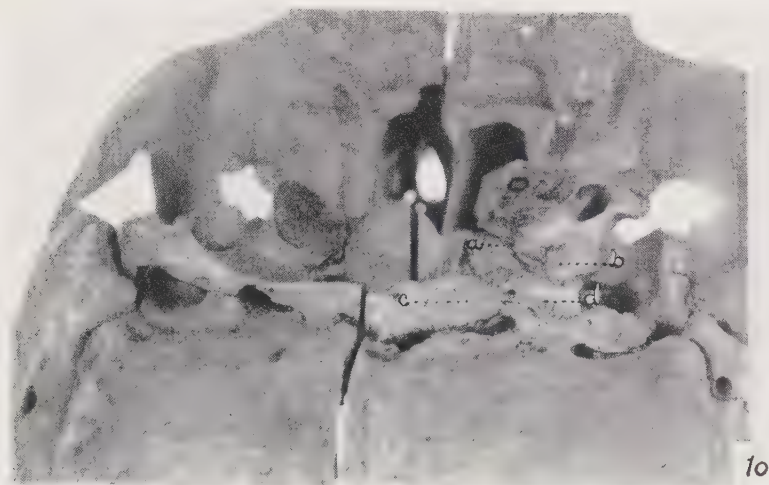
PLATE 3

EXPLANATION OF FIGURES

10 Posterior view of the base of the cranium with the ossicle in place. a, articulation between the ossicle and the atlantal vertebra; b, rudimentary posterior arch of the ossicle; c, posterior arch of the atlantal vertebra; d, groove for the vertebral artery.

11 Anterior view of the epistropheal vertebra with the ossicle in place. a, lateral mass of the ossicle; b, articulation between the ossicle and the epistropheal vertebra; c, body of the epistropheal vertebra.

12 Left postero-lateral view of the base of the cranium with the ossicle and the epistropheal vertebra in place. a, groove for the vertebral artery; b, articulation between the ossicle and the atlantal vertebra; c, rudimentary posterior arch of the ossicle; d, articulation between the ossicle and the epistropheal vertebra.



THE CHANGES IN THE VAGINAL SMEARS
AND ASSOCIATED CYCLIC PHENOMENA
IN THE LOWLAND GORILLA
(GORILLA GORILLA) ¹

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TWO PLATES

Yerkes and Yerkes ('29) in summarizing the literature on menstruation in the gorilla state that, although menstruation doubtlessly occurs in this form as in the other anthropoid apes, it has never been established by creditable observation. In view of this fact and the close phylogenetic relationship of the gorilla to man and the other primates, it seemed that a study of the sexual cycle as well as an examination of the vaginal contents of this form, for cyclic variations, would be of value. The precise mechanism regulating the changes in the vaginal lumen contents and the relationship of these changes to the sex cycle have not been completely clarified for any animal.

The present study was undertaken to describe and attempt to analyze the vaginal contents of the gorilla and to relate the results of these observations to other manifestations of the sexual cycle. This is the first case in which a female gorilla of known age has been observed up to the time of menarche and through several of the menstrual cycles.

This investigation was carried on at the New York Zoological Park and at the biological laboratory of Washington Square College, New York University. I wish to acknowledge

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my indebtedness to my father, the late Dr. Charles V. Noback, who started this investigation, and to the New York Zoological Society for making material available. I also wish to thank Dr. Harry A. Charipper for many helpful suggestions and criticisms during the elaboration and completion of this study.

MATERIALS AND METHODS

The subject of the present investigation is Janet Penserosa, a female lowland gorilla (*Gorilla gorilla*), in the New York Zoological Park. This gorilla has been living continuously at the Park since her arrival from the Belgian Congo on October 31, 1928. At that time, an examination of the carpal bones, dentition and other physical factors indicated that her age was approximately $1\frac{1}{2}$ years (Noback, '30). The animal continued in good health, passing successfully through certain minor ailments with no after effects until September 1935, when a bilateral partial paralysis was observed involving the hind limbs. Slight improvement was noted in this mild condition which has been suspected, although never confirmed, as anterior poliomyelitis. As far as can be determined, this condition has had no apparent effect upon the sexual behavior of the animal.

The life history and other general data have been kept for this gorilla since she arrived in this country. The present study, however, deals more specifically with observations made over a period of approximately 18 months, beginning in January 1936 and extending through June 1937. At the beginning of the period, the gorilla was about 8 years and 10 months old, and at the termination of the study had reached 10 years and 4 months. The data collected for the present study included vaginal smears, rectal and vaginal temperature determinations, measurements of the uro-vaginal cleft, photographs and observations of the external genitalia and a study of the general behavior of the animal. The daily vaginal smears were made over a period of four menstruations, but only three menstrual cycles. The observations on the length of the sex cycles were continued over a longer period.

The gorilla was kept in her own ward and, after becoming adjusted to handling, the procedure of making the smears proved relatively simple. In obtaining the smears, a small wooden spatula was gently inserted into the vagina and slowly withdrawn in such a manner as to avoid excessive friction. The subject was generally quiet during this entire procedure. The technique for fixing and staining the slides is the same as that described by Papanicolaou ('33).

The series of smears were then carefully examined and, by means of an ocular counting chamber, cell counts were made to determine the number of different cell types per smear area. For the purpose of counting, all cells whose cytoplasm stained purple, or shades of purple-eosin, were considered as superficial cells. Those cells which stained eosin, or shades of eosin-purple, were classified as cornified cells. Separate graphs were then constructed for the following cell types: 1) total epithelial cells; 2) superficial epithelial cells; 3) cornified cells; 4) leucocytes. This classification of the cell types is similar to that described by Papanicolaou ('33). In addition, graphs were made on the rectal and vaginal temperature determinations.

One of the most easily measured and yet consistently variable cyclic changes in the external genitalia was the linear dimension of the uro-genital cleft. This measurement was considered to be the distance between the most dorsal point of the muco-cutaneous junction of the vulva and the dorsal surface of the clitoris at its base. In the present investigation, these measurements have been related to the different periods of the menstrual cycle and constitute the basis for another interesting and most useful graph.

The dimensions of the uro-genital cleft, together with certain changes in the vaginal smears, have been used as criteria for dividing the menstrual cycle into periods as follows:

1. *Menstrual phase.* Characterized by the presence of numerous erythrocytes in the vaginal smear and an extremely reduced uro-genital cleft.

2. *Post-menstrual phase.* Characterized by an enlarging uro-genital cleft and with an abundance of leucocytes in the smear.

3. *Copulation phase.* Marked by an enlarging uro-genital cleft and a low leucocyte smear count.

4. *Ovulation phase.* The uro-genital cleft at a maximum and an extremely large amount of mucus in the smear.

5. *Pre-menstrual phase.* The uro-genital cleft becoming smaller (detumescence) and a gradual reduction in the epithelial cells of the smear.

OBSERVATIONS

General. The first observed menstruation of Janet Penserosa was on March 29, 1936. This initial indication of catamenia was probably the date of menarche. After this menses, there followed a sequence of nine periodic menstruations which were characterized by the presence of a bloody discharge on the external genitalia. The elapsed time, from the first day of the initial menstruation until the first day of the ninth menstruation, covering a period of eight sex cycles (from March 29, 1936 to May 3, 1937) totaled 391 days.

The average length of these eight sex cycles was 49 days. Included in the calculation of this average were cycles that ranged from 36 to 72 days. Two of the sex cycles, which occurred during the summer were quite extended (60 and 72 days). Completely omitting these extended periods from the calculations gives an average of 43 days per cycle. There are indications that the spans of 60 and 72 days may represent two complete cycles each, rather than one. If the 60- and 72-day spans are each representative of two cycles, then the average, calculated on a total of ten complete cycles, would be only 39 days.

The length of flow, in each of the eight menses, was 3 days. The ninth catamenial flow was limited to 1 day. This took place in August at a time when primates, in general, exhibit scanty flow and occasionally none at all.

Changes in external genitalia. Interesting changes in the external genitalia were apparent during the time of observation. During the menstrual phase, the labia majora, the labia minora, and the fossa navicularis were shrunken and drawn.

Throughout the postmenstrum and copulative phases, the vulva enlarged and became swollen and full until it reached a maximal size in the ovulatory phase of the sex cycle. In this later period, the external genitalia were greatly enlarged and swollen. Following this phase, detumescence of the vulva occurred. The external genitalia returned rapidly to the shrunken condition observed in the menstrual period. No signs were observed of external swelling and detumescence of the perineal area, so common in the chimpanzee and baboon.

Another interesting and most useful change in the external genitalia is the variation occurring in the linear dimension of the uro-genital cleft. As described previously, this measure is the distance between the most dorsal point on the muco-cutaneous junction of the vulva and the dorsal surface of the clitoris at its base. The minimal length of this cleft always occurred at the time of menstruation. At this time, in Janet Penserosa, the cleft was 1 cm. long (see graph, fig. 7). Immediately following menstruation or a few days thereafter, the cleft enlarged to $1\frac{1}{2}$ cm. Throughout the postmenstrual and copulative phases of the sex cycle the external genitalia and the uro-genital cleft, as well, continually enlarged. On each succeeding day during these phases, the cleft length either remained constant or lengthened. Only one exception was observed. This occurred from the fourth through the tenth day of the 60-day cycle. During this long span the cleft detumescenced to the minimal 1 cm. length, after which it again increased in size. Menstruation did not occur. The average length of the uro-genital cleft during the postmenstrual phase was 1.6 cm. and during the copulative phase it was 2.3 cm. long.

The ovulatory phase was defined as the period during which the vaginal cleft remained at its maximal size. This maximal size for this animal was 3 to 4 cm., which length was maintained for 3 to 8 days. In addition, it is interesting to note that during this ovulatory phase, the animal was irritable and uneasy and would utilize every opportunity to attract attention. She frequently was observed manipulating her genitalia.

The commencement of the process of detumescence of the external genitalia, from their maximal size during the ovulatory phase just described, marked the beginning of the premenstrum. This period varied from 4 to 10 days and was characterized by a rapid shortening in the length of the urogenital cleft from the maximal length to the minimal length (graph, fig. 7).

Variation of the uro-genital cleft length was observed for 2 months before initial menstruation.

General nature of the variations of the vaginal smears during the sex cycle. During menstruation, the total epithelial cell counts were lowest while the superficial cells with their nuclei in various stages of pycnosis dominated the picture. Totally cornified, non-nucleated eosinophiles, were few in number. Decidual cells were never seen and profuse bleeding never occurred during the menstrual phase of the cycles studied although erythrocytes were always present during the 3-day menstrual period. Once the maximum of total epithelial cells was attained, at the beginning of the copulative phase, it was maintained until the following premenstrum, at which time the count went down to the low number observed in the menstrual phase. Superficial cells were the most common while cells intermediate between the true superficial cells and the cornified cells became more prominent as the postmenstrual phase progressed. During the copulative phase, superficial cells with pycnotic nuclei and lightly staining cytoplasm was abundant while masses of cornified cells and superficial cells varied greatly during the ovulatory phase, at which time the total cell counts were highest of any time during the entire sex cycle. Following ovulatory phase, the total epithelial cell counts dropped during the premenstrual phase as superficial cells became more common than cornified cells. The cornified cell and epithelial cell counts varied throughout the entire sex cycle. A rise in the number of cornified cells was coupled with a lower superficial cell count. However, at no time did the cornified cell counts exceed the 'superficial cell' count.

All intergradations on the leucocyte numbers, from leucocytosis to complete leucopenia, were observed at some time during each sex cycle. All phases blended into each other. Therefore, it was difficult to define the stages of the sex cycle on the basis of the vaginal smears alone. The menstrual phase was characterized by the presence of erythrocytes and a relatively high number of leucocytes, mainly polymorphonuclear leucocytes. The leucocyte counts tended to increase during the postmenstrual phase until a high point was reached at the end of the phase when a pronounced leucocytosis occurred. During the copulative phase of 4 to 9 days' duration, the leucocyte counts were low. In the following ovulatory phase there was an extreme variation in the leucocyte numbers. Leucopenia was observed in three of the five phases studied. Similarly, in four of the five premenstrual periods, leucopenia was characteristic.

Basal cells were occasionally seen but no cyclic activity of these cells could be discerned.

Although a cyclic activity of the superficial cells was recognized, the desquamation was continuous and rather uniform during the main portion of the intermenstrum. The presence of leucocytes, a low epithelial cell count, and a high percentage of superficial cells in the vaginal smears of the mid-interval of the 60-day summer cycle seem to indicate that, during the middle of this cycle, Janet Penserosa may have been experiencing a physiological condition similar to menstruation. This observation is the basis for the contention that the 60-day span may, in reality, be composed of two sex cycles.

Temperature changes in the sex cycle of the gorilla. The rectal temperature varied from 98°F. to 100 $\frac{1}{5}$ °F. The vaginal temperature varied from 98°F. to 100 $\frac{3}{5}$ °F. As was expected, the rectal and vaginal temperatures fluctuated together. However, no definite relation between the temperature and the phases of the sex cycle could be found.

DISCUSSION

General. Grabowsky ('04 and '06) described signs of sexual activity in the gorilla 'Puissi,' when she was a little over the estimated age of 5 years. Yerkes and Yerkes ('29) doubt whether Grabowsky's observation is acceptable as proof of menstruation in the gorilla. They state that there is adequate ground for believing that the female chimpanzee does not ordinarily achieve sexual maturity before 8 years of age. On this basis and because of the close relationship between these two apes, they feel that it is improbable that the gorilla reaches maturity at an age younger than that of the chimpanzee. The observation of Yerkes ('28) on the gorilla 'Congo,' which presumably was just over 7 years of age and did not exhibit the phenomenon of menstruation, may be considered to throw additional doubt on Grabowsky's observation.

The literature on menstruation in the gorilla is summarized by Yerkes and Yerkes ('29) who claim that the occurrence of menstruation in the gorilla is not disproved, but that no dependable evidence for the occurrence of this phenomenon is available. They state that definite descriptions of the appearance of menstruation and its periodicity are lacking from the literature. They conclude that, although menstruation doubtless occurs in the gorilla as well as in the other anthropoid apes, the fact has never been established on the basis of creditable prolonged observation. The present work supplies these observations.

Gross evidence, based on the presence of a bloody discharge on the external genitalia, is reported for 3 days from March 29th to 31st on Janet Penserosa (Noback, '36). In the present study, the examination of the vaginal smears, taken from the same animal from February 10 to March 28, 1936, show no erythrocytes. All evidence, from the study of the vaginal smear contents and the gross observations of the external genitalia, did not give any indication of menstrual flow previous to March 29, 1936. This date, therefore, has been set as being, in all probability, the date of menarche in the gorilla,

Janet Penserosa, at which time her age was determined as being 9 years and 1 month. Recalling that the average date of menarche in the human is 13.5 years (Engle and Shelesynak, '34), then this phenomenon in Janet Penserosa presents evidence indicating an earlier date of puberty for the gorilla.

The length of the sex cycle on the gorilla agrees in essentials with the cycle length in the other higher primates. The average length in days of the sex cycle, for the other higher primates, varies from the 27- to 28-day cycle of the *Macacus rhesus* (Corner, '23; Hartman, '32), the 31.4- to 35-day cycle of the baboon (Zuckerman and Parkes, '32; Gillman, '37), the 32-day cycle of the orang-utan (Aulmann, '32), the 28-day cycle of the human (Bartelmez, '37) to the 35.5- to 36-day cycles of the chimpanzee (Elder and Yerkes, '36; Tomilin, '36). All these figures are averages, derived from many cycles, which have a fairly wide range of variation with respect to the time in days.

In the present work, the 49-day cycle average for the eight observed menstruations of Janet Penserosa may probably prove too long to be considered for the true modal length for the sex cycle in the gorilla. An average sex cycle length of 39 days for the gorilla may prove nearer to the modal length and may be justified by accepting all the manifestations of menstruation which were observed in the vaginal smears in the mid-period of the two long summer spans as indicating that each of these long periods represent two cycles instead of one. Therefore, the calculation for the average sex cycle length in the present study could be based on ten cycles instead of only eight. All these data on the sex cycle lengths, including the gorilla, are interesting in that they show that the time span of the cycles in the various higher primates fall within comparable limits.

Changes in the external genitalia. The external genitalia in the gorilla undergo a cyclic activity that is comparable to that of the other higher primates. Russell and Zuckerman ('25) report a circumgenital swelling of the external genitalia

that recurs regularly with menstruation in the *Macacus* monkeys. Pocock ('25) observes genital swelling in the baboon as did Zuckerman and Parkes ('32). The latter worker describes the vulval and circumanal tissues of the baboon as quiescent during menstruation, swelling to a maximum up to shortly before the middle of the sex cycle, after which detumescence of the sex skin begins and continues until quiescence is reached. Gillman ('37) confirms these findings and adds that ovulation occurs at some time during the period of maximal swelling. A similar condition of a cyclic activity of the sex skin in the chimpanzee is reported by Elder and Yerkes ('36).

Activity of the circumanal region of the gorilla, Janet Penserosa, was slight and did not compare in intensity with the sex skin activity of the baboon (Zuckerman and Parkes, '32; Gillman, '37), and of the chimpanzee (Tinkelpaugh, '33 and Elder and Yerkes, '36). However, the lengthening and shortening of the uro-genital cleft is comparable in its cyclic activity to the swelling and detumescence of the sex skin of the chimpanzee and the baboon.

In regard to the variation of the uro-genital cleft, it is interesting to note that several months previous to menarche in the gorilla variable changes occurred in its linear dimensions, presenting a situation very much similar to that described by Tinkelpaugh ('33) in the chimpanzee. In this case he found changes in the sex skin previous to menarche.

Vaginal smears. There is a definite cyclic variation in the epithelial cells during the various phases of the sex cycle in the gorilla. These vaginal mucosal cells are least numerous during the menstrual phase and most abundant during the intermenstrum. This cyclic activity is similar to that of the other primates (Corner, '23, and Allen, '27 on the rhesus monkey; Zuckerman and Parkes, '32, and Gillman, '37 on the baboon; Tinklepaugh and Van Campenhout, '31 on the Chimpanzee; and Papanicolaou, '33 on the human). There is no indication of a massive desquamation of the epithelial elements from the vaginal mucosa although cyclic activity

may be inferred from the smear contents. This agrees with Smith and Brunner ('34) who note gradual but never massive desquamation in the human.

The presence of leucocytes in the smears during the menstrual and postmenstrual phases is similar to the results obtained on studies of the vaginal smears of the other primates. In the period following postmenstrum the leucocyte counts varied. The exact significance of the leucocyte variation is unknown to the investigator.

The so-called typical smears for forms which have been studied extensively, as described in the literature in terms of a numerical evaluation of the different cell types present must be remembered as being, in reality, the result of an average obtained after extensive observations on many different specimens or in many samplings of smears in a good number of cycles. An examination of the data upon which these averages were calculated discloses a great variation in the numbers of each type of cell which may be met in the typical periods of the cycle. The data collected for the results of the examination of the smears in the gorilla are tenable and comparable to the results thus far reported for other primates.

SUMMARY

1. The first definite menarche ever to be observed in a lowland gorilla (Janet Penserosa) was found to occur at the beginning of the ninth year of age.

2. Nine menses and eight complete menstrual cycles have been noted and correlated.

3. The cycle has been shown to average 43 days in length with a possibility of a 39-day cycle being the true average for the animal described in the present report.

4. The cycle has been divided into five phases with the menstrual phase showing the least variation and maintaining a 3-day period.

5. Changes in the linear dimension of the uro-genital groove are described and related to different phases of the cycle. The measurement is greatest during the ovulatory phase and smallest during the menstrual phase.

6. Cyclic changes in the nature and cellular content of the vaginal smears are similar to those reported for other primates including man.

7. No correlation could be determined for rectal and vaginal temperature variation and the different phases of the menstrual cycles observed.

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ABBREVIATIONS

CL, clitoris	MC.J., dorsal point of the muco-cutaneous juncture
L.M., labia majoria	U.G.C., uro-genital cleft

PLATE 1

EXPLANATION OF FIGURES

1 Outline drawing to show the anatomy of the external uro-genital region of the gorilla.

Photographs of the uro-genital region of the gorilla to show variations in the linear dimension of the uro-genital cleft during the phases of the sex cycle in the gorilla, Janet Penserosa.

- 2 The menstrual phase.
- 3 The postmenstrual phase.
- 4 The copulative phase.
- 5 The ovulatory phase.
- 6 The premenstrual phase.

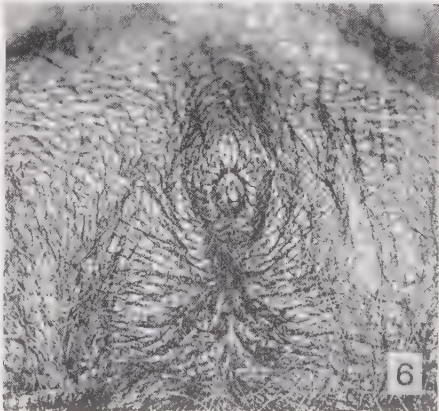
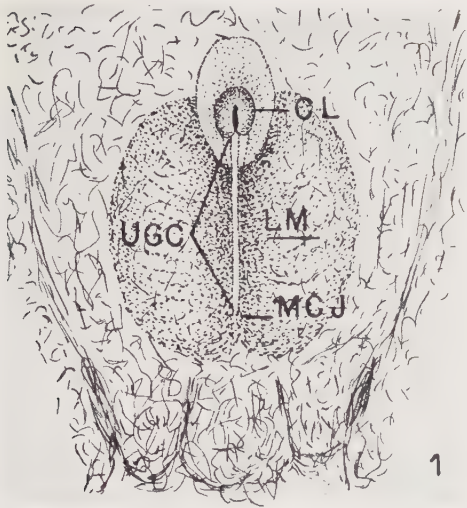
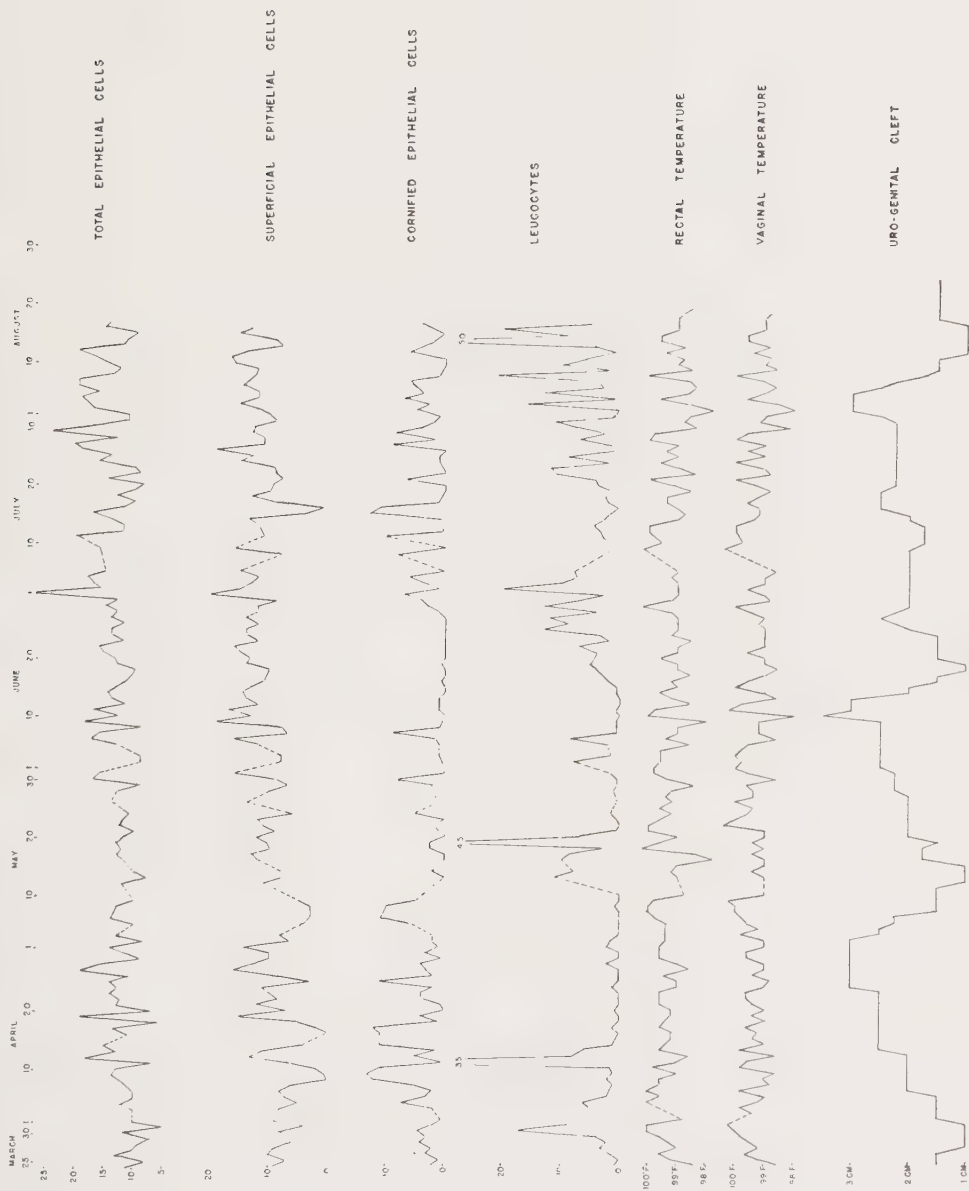


PLATE 2

EXPLANATION OF GRAPHS

The graphs in this plate give data for the sex cycle of the gorilla from March 24th to August 19th. Menstruation dates during this time were:
1) March 29th, 30, 31st; 2) May 12th, 13th, 14th; 3) June 17th, 18th, 19th;
4) August 17th.

- 1 Graph showing variations in the number of total epithelial cells in the vaginal smears of the gorilla.
- 2 Graph showing variations in the number of superficial epithelial cells in the vaginal smears of the gorilla.
- 3 Graph showing variations in the number of cornified epithelial cells in the vaginal smears of the gorilla.
- 4 Graph showing variations in the number of leucocytes in the vaginal smears of the gorilla.
- 5, 6 Graphs showing variations in the rectal and vaginal temperatures (in degrees Fahrenheit).
- 7 Graph showing variations in the length of the urogenital cleft (in centimeters).



THE LYMPHOCYTES IN RELATION TO ERYTHROCYTE PRODUCTION

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Since the time of Bizzozero's (1890) original monograph, the bone marrow of birds has been a favorite subject for investigations of the details of hemocytopoiesis. Contemporary and subsequent early workers in this field with similar material include Denys (1887), van der Stricht (1892), Dantschakoff ('09) and Venzlaff ('11). The current concept of the primary origin of erythrocytes and leucocytes is based on the conclusions of Doan, Cunningham and Sabin ('25), drawn from their studies of experimentally depleted marrow of pigeons. The salient feature of this concept concerns the origin of the erythrocytes from endothelial cells of intersinusoidal marrow capillaries. Acceptance of this alleged fact necessarily implies rejection of the monophyletic doctrine of blood-cell origin.

With so much expert microscopical attention to avian bone marrow over a period of half a century it seems almost incredible that the one most important and most pertinent feature should have been either overlooked or ignored; namely, the presence in the bone marrow of considerable numbers of lymphoid nodules. This is all the more surprising since these nodules supply definite evidence respecting the hemogenic significance of the lymphocytes. The solution of the problem of the origin of erythrocytes is fully patent in the very structures in avian bone marrow which were given little or no attention in these earlier investigations. Moreover, this material supplies at least a major portion of the answer to the perennial query concerning the function of the lymphocytes. Here is the most favorable material for the solution

of two of the most vexing problems in normal hematology—and it has escaped recognition for 50 years, except for brief mention by Venzlaff.

As is well known, birds lack lymph nodes; these structures occur first in mammals. What needs emphasis in this connection is the fact that compensation for absence of lymph nodes is accomplished in birds by the inclusion of much lymph-nodular material in the bone marrow, especially that of the femur and the tibia. Here lymphoid and myeloid tissues constitute a common blood forming organ. The condition is comparable to that of the hemopoietic organ, the spleen, of lower vertebrates, e.g., cyclostomes and lungfishes (Jordan and Speidel, '30, '31). These hemogenic organs supply the proper material for investigation of the possible genetic relationship between lymphocytes and definitive blood cells: erythrocytes, monocytes and granular leucocytes.

Consideration of the phylogenesis of blood forming tissues (Jordan, '33), omitting details and minor variations, reveals the following essential facts: The spleen constitutes the primary erythrocytopoietic organ from cyclostomes to urodeles. In anurans the bone marrow becomes transiently active, following metamorphosis and hibernation. In reptiles, spleen and bone marrow share in variable proportions in red cell production. In birds the red marrow becomes in the adult the exclusive hemopoietic locus. In mammals also red-cell production is normally restricted to the bone marrow. In mammals the original threefold function of lymphocytopoiesis, granulocytopoiesis and erythrocytopoiesis of the primitive spleen, as represented in cyclostomes, becomes apportioned among lymph nodes, spleen and bone marrow. In a certain sense the spleen of mammals represents a vestigial organ.

Note should here be made of these facts: In higher fishes and amphibian tadpoles the kidneys (mesonephros and pronephros respectively) constitute a variable but important erythrocytopoietic organ supplementary to the spleen. In urodeles generally granulocytopoiesis is practically restricted to the subcapsular region of the liver; in selachii, to the sub-

capsular and intertubular stroma of the gonads. In certain urodeles, e.g., Proteidae, the intertubular stroma of the mesonephros is very active in granulocyte formation.

The central fact in the phylogenesis of hemopoietic tissue concerns the small lymphocyte: Both in the erythropoietic and granulocytopoietic loci, the small lymphocytes, daughter cells of larger lymphocytes (hemocytoblasts), grow to the size and acquire the general features of the mother large lymphocytes, and then differentiate into erythrocytes within venous sinuses, into granulocytes in the intervacular stroma. The evidence from studies of hemogenesis in amphibians is especially clear and convincing with respect to the erythrocytogenic capacity of the small lymphocyte.

It seems a matter of some significance that investigators in this field who approach these problems from the standpoint of comparative hematological data, chiefly zoologists and anatomists, arrive at conclusions in accord with the monophyletic theory of blood formation and the embryonal polyvalent nature of the small lymphocytes; while those who confine their interests to mammalian materials, chiefly clinicians, more generally favor the polyphyletic theory of hemogenesis and the concept of the definitive specialized constitution of the lymphocytes. It is clear, then, that the problems of the origin, function and fate of the lymphocyte cannot be separated from the problems concerning the origin and genetic relationships of the several varieties of blood cells.

Proceeding to the description of conditions in the lymphomyeloid marrow of birds, as represented especially in chicken, pigeon and turkey, we note that next to the presence of the lymphoid nodules the most striking feature concerns the variable aggregation of lymphocytes in the peripheral, subendosteal area. Before discussing the significance of this incomplete envelope of lymphoid tissue, attention must be directed to the variable condition of the marrow at different levels of the active myeloid cylinder of long bones.

At certain levels the otherwise hemopoietically active marrow is in hypoplastic condition; blood moves freely from

central arteriole through radial arterial capillaries to the subendosteal vascular rete and is returned through open venous sinuses to the central medullary vein. Adjacent to such areas are regions of moderate 'hyperplasia.' Here the venous sinuses are filled at their peripheral ends with predominantly large lymphocytes (hemocytoblasts). There is here some degree of leucostasis. The central ends of these venous sinuses contain almost exclusively approximately definitive erythrocytes. In the peripheral leucostatic areas occur various stages in the maturation of erythrocytes from hemocytoblasts. There is no evidence of endothelial activity in the formation of ancestors ('megakaryoblasts') of red cells; the enclosed hemocytoblasts represent hypertrophied small lymphocytes. The intervacular stromal areas contain variable numbers of similar lymphocytes and various stages in the development of granulocytes.

Adjacent to the moderately active areas are regions of lymphoid hyperplasia. The predominating cell is a small lymphocyte. Large and medium-sized lymphocytes occur in variable numbers, either scattered among the small lymphocytes, or more or less sharply aggregated at one side, or aggregated centrally in the form of a genuine germinal center. Only the large and medium-sized lymphocytes proliferate; the smaller lymphocytes show a high degree of motility.

In such lymphocytopoietic areas the venous sinuses are collapsed and practically unrecognizable. An occasional vessel of capilliform character becomes discernible by reason of a long sharply demarked single or double line of enclosed small lymphocytes. Subjacent to such compact lymphoid areas the venous sinuses are dilated with growing lymphocytes, the majority of hemocytoblast size and cytology. In these leucostatic regions red-cell maturation is very active, proceeding in the direction from endothelial wall to axis of the vessel. The axial region usually contains a few definitive erythrocytes. There is not the slightest evidence of endothelial participation in the production of hemocytoblasts. The hemocytoblasts are simply enlarged small lymphocytes.

The central ends of these peripherally leucostatic sinuses are generally open and more or less completely filled with definitive erythrocytes. The evidence is clear that in these areas of compact lymphoid tissue, small lymphocytes are produced by the proliferation of large and medium-sized lymphocytes, and that the small lymphocytes migrate very actively both into the venous sinuses and into the intervascular stroma where, following a certain amount of growth, they differentiate respectively into erythrocytes and granulocytes.

An important variation must be noted to this more general condition in these hyperplastic lymphoid areas; immediately subjacent may be a variable number of completely collapsed venous sinuses, the result of the stoppage of blood-plasma flow peripherally due to hemocytoblastic stenosis. A striking feature of these collapsed sinuses is their thin envelope of small lymphocytes. These lymphocytes almost without exception are covered with very conspicuous stout pseudopods, indicating motility. These small lymphocytes have migrated from the superjacent lymphoid tissue and are apparently making an effort to enter the collapsed sinuses. Following maturation of the stenosing hemocytoblasts to erythrocytes in the peripheral ends of the sinuses the blood current is re-established, plasma enters the collapsed central ends and, after the resulting dilation and consequent stretching of the endothelial wall, the enveloping small lymphocytes pass through the now less resistant endothelial barrier to enter the blood stream. The persistent intervascular stromal lymphocytes, grown into hemocytoblasts, differentiate into granulocytes and only subsequently migrate into the blood vessels.

The intersinusoidal venous capillaries also play a variable role in erythrocytogenesis. They provide loci for the multiplication and maturation of hemocytoblasts into erythrocytes. In such condition they are especially conspicuous in the early stages of restoration following experimental depletion (Jordan and Johnson, '35). In most striking form they occur most numerous in the intermediate marrow region.

One may encounter longitudinal sections of long length of such capillaries, in which hemocytoblasts have become lined up in close single file. Such appearances have suggested the erroneous interpretation of endothelial origin of the erythrocyte ancestors ('megakaryoblasts' of Doan, Cunningham and Sabin, '25). These narrow intersinusoidal capillaries are favorable places for the accumulation of cohesive lymphocytes, for growth into hemocytoblasts, for proliferation, and for maturation into erythrocytes. In these progressive conditions the vessels become expanded and represent the erythrocytic capillaries of earlier authors.

The compact sharply circumscribed lymphoid nodules have essentially the same structure and function above described for the peripheral irregular collection of compact lymphoid tissue. The nodules occur more generally in the subendosteal area; but may occur even near the axis. Detailed study of these nodules reveals certain important additional facts. These nodules have a cyclic function; they grow from small beginnings, attain a maximum size of approximately 300 μ , become drained of their cellular content, and disappear. Their original site is marked by a relatively small nodule of reticular tissue. Subsequently a new cycle is begun by the collection of a few small lymphocytes, either centrally or in the margin of the connective tissue nodule. These small lymphocytes grow in size, produce a germinal center, proliferate to form more small lymphocytes and become aggregated as a spheroidal typical lymphoid nodule.

Adjacent to such nodules one can usually find an arteriole. This vessel contributes a mesh of capillaries to the full-grown nodule. Over the periphery of the nodule the capillaries empty into venous sinuses, disposed radially with respect to the central nodule. The small lymphocytes migrate centrally into the capillaries and peripherally into the intervacular stroma. Within the capillaries the small lymphocytes are swept into the extranodular sinuses. The vast difference in caliber between the nodular capillaries and the extranodular sinuses produces a sudden and decided slowing of the blood

current in the nodular ends of the sinuses. Under these conditions the small lymphocytes collect, cohere, hypertrophy and, as hemocytoblasts, occlude this portion of the venous sinuses. In these transiently leucostatic sinuses the hemocytoblasts mature into erythrocytes, in an endothelio-axial sequence. There is no sign of endothelial erythrocytogenic activity. A second and more rapidly effective method of mass lymphocyte transfer to the venous sinuses is by process of reticular cell transformation into endothelium, continuous with that of the capillary mesh. In this way large collections of lymphocytes become incorporated within the vascular channels, essentially as described by Venzlaff ('11). This portion of the intranodular vascular system thus constitutes a true reticulo-endothelium. The intervascular stromal lymphocytes differentiate into granulocytes.

Emphasis on the lymph-nodular mode of origin of blood cells in avian marrow does not imply complete restriction of hemocytoblast production to compact aggregation of lymphocytes. The reticular stroma of any region of the marrow is a potential source of origin of hemocytoblasts. This potentiality is widely expressed, especially in hyperplastic marrow. The hemopoietic reticular cell activity is more evident in regions of early hyperplasia, immediately following natural local or experimentally induced widespread hypoplasia. The original reticular cell derivative more frequently has the nuclear features of a typical hemocytoblast. The proliferation products of these larger hemocytoblasts have the nuclear features of more typical small lymphocytes. Accordingly, whether nodular stroma or general stroma is under consideration in the processes of hemogenesis, typical small lymphocytes, as derivatives of reticular cells, are involved in the processes of erythrocyte and granulocyte production.

The foregoing leads logically to consideration of the question of the significance of the small lymphocyte. This question presents several aspects: 1) relation of lymphocytes to reticular connective tissue; 2) fate of lymphocytes in the mammals poured from thoracic duct into the blood stream

and 'lost' in enormous numbers daily; 3) possible relation of lymphocytes to erythrocyte, granulocyte, monocyte and thrombocyte production.

Histological evidence indicates that the original lymphocytes represent reticular (or mesenchymal) cells which have separated from the primary syncytium and retracted their processes with the assumption of a spheroidal form. Corresponding to size variations among reticular cells the lymphocyte derivatives are divisible into three chief categories: small, medium-sized and large. These size variations are maintained collectively as the result of growth and proliferation. Whether lymphocytes may reverse this process and become connective tissue cells (fibroblasts, e.g., in scar tissue) as is widely maintained (e.g., Carrel and Ebeling, '22) need not be further discussed in this connection.

However, on the basis of a differential reaction to vital dyes of the reticulum cells and the lymphocytes of lymph nodes, Aschoff ('24) doubts the possibility of a transition from one to the other and insists on a definite distinction between these cells. But, in the case of the spleen he accepts a close genetic relationship between the reticulum cells and the lymphoid pulp cells (parenchymatous cells, splenocytes). In the spleen the so-called splenocytes of the pulp also absorb vital dyes. The occurrence of morphologically transitional stages between lymphocytes and monocytes (splenocytes, macrophages) in the spleen shows that at least a portion of the dye-storing mononuclear leucocytes derive from lymphocytes. And the studies of Bloom ('28) and Conway ('38) on experimentally induced hypermonocytopoiesis show conclusively that lymphocytes may become transformed into monocytes. The same conclusion is indicated by the results of Downey's ('17, '18) experiments with static blood in ligated segments of veins; and by those of Jordan ('25) with frogs injected with India ink. Accordingly, the fact that lymphocytes as such under the usual conditions are storage-negative as regard vital dyes may only mean that they have not within the imposed time limits been able to express, in response to

the specific stimulus, their phagocytic or resorptive potentiality. It may be noted further that the reticulo-endothelial substratum of red bone marrow is quite generally accepted as the primary source of the blood cells. As a reticular-cell derivative the lymphocyte would necessarily constitute a second early step in the maturation of blood cells. And as a constituent of the circulating blood it should logically be regarded as the circulating portion of the otherwise stationary reticulo-endothelial system.

We face, then, the second aspect of our larger question, namely, the fate of the lymphocyte of the circulating blood. Yoffey ('33) has recently confirmed the earlier conclusion of Davis and Carlson ('09), Rous ('08) and Bunting and Huston ('21) that more lymphocytes are lost daily in the body of the mammal than are present at any one time in the circulating blood. Yoffey summarizes his experiments on the dog with the statement that the lymphocytes are replaced two and one-half times every 24 hours. Translated for man this datum would mean that approximately twenty-five billion lymphocytes vanish somewhere in the human body daily. What happens to this enormous number of 'lost' lymphocytes? Bunting and Huston ('21) suggested that in the rabbit they pass through the intestinal mucosa and disappear with the feces. We ('23) have previously shown that this route of destruction accounts for the loss of only a negligible proportion under normal conditions. Sjovall ('36) makes the claim that they migrate from the capillaries, especially in the subcutaneous tissue, into the tissue spaces and then into capillary lymphatic radicles; that they are not actually lost from the body but simply traverse a circuit between blood and lymphatic channels. If such were the fact, lymphocytes would have to pile up in the tissues, which is not normally the case. Finally, it may be noted that there is no evidence of any but a very minimal amount of lymphocyte disintegration in the blood stream.

The question remains: What happens to the approximately twenty-five billion lymphocytes which are daily poured into

the blood and daily apparently leave the body? The answer is supplied by the demonstration that in the bone marrow of the bird lymphocytes function as ancestors of erythrocytes and leucocytes; in connection with the fact that in active marrow generally, including that of birds and mammals, the subendosteal region over considerable areas is predominantly lymphoid. Putting these two circumstances together the strong presumption follows that the 'lost' small lymphocytes are filtered out of the blood stream in the subendosteal sinusoidal rete where they undergo growth and assume the function of precursors of erythrocytes. It may be noted that the relation of the subendosteal venous channels to the long radial arterial capillaries is mechanically ideal for the suspected filtering process; the sudden transition of the very narrow capillary lumen to the relatively enormous lumen of the venous sinus effects an abrupt, almost complete stasis, under which most favorable conditions the cohesive lymphocytes can collect and hypertrophy and produce a stenosis of certain venous channels, where the maturation of erythrocytes can be consummated in the so-called erythrogenic capillaries, as described above for the marrow of the chicken.

The total number of erythroplastids in the human body is approximately twenty-five trillion. Taking the life tenure of the erythrocyte at its maximum, approximately 100 days, at the least 250 billion erythrocytes must be replaced daily. When one examines sections of even the most active normal red marrow one is surprised at the relatively small number of cells in mitosis. It seems improbable that compensation for the daily natural red-cell disintegration could be effected homoplastically. This discrepancy can be at least logically satisfied by the daily addition to the bone marrow of the billions of 'lost' lymphocytes as potential erythroblasts.

The answer to the third aspect of our general question regarding the significance of the small lymphocytes was largely implied in the answer to the two other aspects. The evidence from the bone marrow of birds demonstrates a direct genetic relationship between the small lymphocyte, as common mother

cell with multiple developmental capacities, and all the other varieties of blood cells: erythrocytes, granulocytes, monocytes and thrombocytes. The evidence for the intravascular differentiation of small lymphocytes, following a certain amount of growth, into erythrocytes, and the stromal differentiation of identical cells into granulocytes need not be repeated. As regards the genetic relationship between lymphocytes and monocytes the evidence seems unequivocal, but whether the process is restricted normally to the stroma, or whether it may also occur to some extent within the blood channels is not clear. Possibly the monocytes of the blood stream should be regarded as migrants from the stromal source of origin.

The occurrence of thrombocytes (spindle cells) in this blood presents a confusing problem and might seem at first consideration to discount fatally our chief contention that the small lymphocytes of avian blood constitute the primary erythrocyte precursors. It is disconcerting that the otherwise so favorable feature of this material for investigation of the question of the genetic relationship of the small lymphocyte to erythrocyte production should be vitiated to some degree by the presence of large numbers of thrombocytes (25,000 to 50,000 per cubic millimeter of blood). Thrombocytes develop from small lymphocytes, apparently exclusively within the venous sinuses; they are only slightly modified small lymphocytes. Except for the generally fusiform shape and their usual content of one or several juxtanuclear collections of azurophilic granules they have the nuclear and cytoplasmic grosser features of small lymphocytes. The suspicion then arises that the small lymphocytes that migrate into the capillary channels of the lymphoid nodules and are swept into the venous sinuses differentiate only into thrombocytes. It is certainly a fact that small lymphocytes do become thrombocytes.

It is also an unequivocal fact that small lymphocytes grow to become large lymphocytes (hemocytoblasts) and that these larger lymphoid cells differentiate within the venous sinuses

into erythrocytes. The conclusion seems to follow that small lymphocytes which meet an effective differential stimulus before there was opportunity for growth become thrombocytes; small lymphocytes lodged in sinuses where conditions for growth were favorable elaborate hemoglobin and become erythrocytes. In support of the central claim of this argument that the small lymphocytes are embryonal polyvalent cells and capable of differentiation into erythrocytes in the expression of one of their multiple developmental capacities, the following facts may be re-emphasized: 1) Evidence of endothelial participation in hemocytoblast origin is definitely and completely negative. 2) While sinuses occur with a mixture of large, medium-sized and small lymphocytes, sinuses can also be seen in which the lymphocytes are practically exclusively either of small, medium-sized or large variety. 3) Abundant transition stages, in closely graded series both in the lymphoid nodules and in the hemostatic sinuses, leave no escape from the conclusion that small lymphocytes grow into medium-sized and subsequently into large lymphocytes. 4) Frequent mitoses of large and medium-sized lymphocytes, both in the nodules and sinuses, demonstrate the homoplastic origin of smaller from larger varieties of lymphocytes.

Finally, the objection might be raised with some pertinency that while the process of hemogenesis in the marrow of birds may be exactly as described above, there is still no certainty that the cells designated as small lymphocytes are actually genuine lymphocytes. This hypothetical position recalls the wish expressed at the symposia on the lymphocyte given in connection with the last two annual meetings of the American Association of Anatomists for a specification of differential criteria for the recognition of genuine lymphocytes. The apparent hopelessness of the situation, especially as regards agreement on whether blood formation proceeds according to the monophyletic or polyphyletic concept, and on the genetic significance of the lymphocyte in hemocytogenesis, may be summarized in an extract from the conclusion of the Chairman of the second symposium: "While it is not likely that

anyone changed his opinions as a result of the discussion and evidence presented” Nevertheless, I venture to define a genuine small lymphocyte as the predominating cell, with variable nuclear, nucleolar and cytoplasmic features, in the cortical nodules and medullary cords of normal lymph nodes. Identical cells in other organs, notably spleen, tonsils, hemal nodes, lymphoid nodules of intestinal mucosa and bone marrow of mammals are also genuine lymphocytes. Homologous cells in hemocytopoietic organs, notably spleen, of lower vertebrates are also true lymphocytes.

In the case of chicken, pigeon and turkey, I have made a careful detailed comparative study of the predominating parenchymal cells of nodules of bone marrow, spleen and rectal ceca. These cells are apparently cytologically identical. If the prevailing small lymphoid cells of the spleen and the nodules of the ceca of birds are genuine small lymphocytes, then the small cells of the lymphoid nodules of the bone marrow are also genuine lymphocytes. Since these same cells in the spleen of the young bird, and homologous cells in the spleen of reptiles, amphibians and fishes, differentiate in part into erythrocytes, it should not be surprising that as a resident of the favorable venous sinuses of the bone marrow the lymphocyte should here also express its potentiality for erythrocytogenesis. Moreover, the identical cell of the marrow nodules of local primary heteroplastic origin should logically have the same capacity.

The objective evidence seems definitive regarding the normal erythrocytogenic function of the small lymphocytes in the marrow of birds. Applying the conclusions built upon the data of comparative hematology, more especially those from hemocytopoietic tissues of amphibians and birds, to the mammalian group the very strong inference is suggested that the small lymphocytes of mammals are also polyvalent embryonal cells which, filtered out in the bone marrow, function as supplementary blood-cell precursors in addition to local ancestors.

All this must be regarded as having significance with respect to the various anemias and leucemias. It might perhaps be held that since effective means are now at hand for the therapeutic control of certain anemias and leucemias, it is of small importance whether the erythrocyte has a lymphoid, an endothelial or a histiocytic origin; or whether the several granulocytes develop from lymphocytes or specific leucoblasts; or even whether the small lymphocyte is an embryonal polyvalent cell or a specialized definitive cell. One may maintain, however, that procedure from correct hemocytologic premises should promise more adequate control of blood dyscrasias than approach from mistaken premises; it would seem of some therapeutic importance whether the erythrocyte is a transformed endothelial cell, a matured specific hemocytoblast or a differentially transformed polyvalent lymphocyte.

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SURVIVAL OF RABBIT EMBRYONIC TISSUES AFTER REMOVAL FROM THE UTERUS AND EXPOSURE TO LOW TEMPERATURE

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ONE PLATE (EIGHT FIGURES)

Body tissues will survive the death of an animal for varying periods of time and under different environmental conditions (compare Alvarez, '37 for a brief review of this subject). Both embryonic and adult tissues have been studied as well as those from warm and cold blooded animals. Numerous factors condition survival, for instance; metabolic rate of the tissue and the animal of which it is a part, temperature at which the tissue is kept after death, contamination, function, presence or absence of blood, amount of dehydration, etc. Various means have been used to determine the survival time, such as physiological, histological and culture in vitro methods.

The blastocyst stage and very young embryos of the rabbit survive isolation and cold temperature for a time as tested by the culture in vitro technique. The same technique has been employed by Nicholas ('34, '38) for the rat embryo which he has found will withstand rather wide limits of temperature variation for some time in Ringer's solution and in a circulating medium. Development is normal in a uterus placed in a subcutaneous pocket. Chick embryonic tissues will likewise survive exposure to low temperatures (Bucciante, '31).

¹ This work was aided by a grant from the Rockefeller Foundation.

In transplantation experiments it is not always possible or convenient to utilize immediately all of the material available. To be able to preserve it several days for future experimentation would prove invaluable. In testing this, as well as the total survival time, young rabbit embryos were stored at 5°C. and transplantation of samples made daily thereafter to the omental bursa of the adult rabbit. The results described in this report seem to indicate that many embryonic tissues of these ages will survive for 2 to 3 days at least at a low temperature and will differentiate in a typical fashion when transplanted.

MATERIALS AND METHODS

Young rabbit embryos of 13 and 15 days of age were used. Either the entire uterus containing the embryos was transferred to a moist, covered dish or the embryos with the membranes and part of the placenta were removed from the uterus. The membranes were then pricked and the embryos placed between moistened layers of cotton in covered dishes and subsequently placed in a refrigerator set at 5°C. Sterile materials were used throughout. Material from several embryos was transplanted to the omental bursa at once and this, together with the results of previous studies, served as controls. At daily intervals thereafter transplantations were made until the reserve embryos were exhausted (about 9 days). Since the presence of blood in the tissues is said to aid survival (compare Alvarez), loss of blood before transplantation was avoided.

The omental grafts were allowed to develop about 10 days and then removed for study. The material transplanted consisted of more or less isolated rudiments of kidney, brain, oesophagus, stomach, eye, intestine, liver, pancreas, trachea, lung, skin and limb bud. These rudiments, with the exception of kidney, have been found previously to differentiate well in omental grafts within the same species. Both male and female rabbits of various ages were used as hosts and the embryonic material was brought slowly to room temperature before transplantation.

EXPERIMENTAL

General graft structure

Superficial examination of the donor embryos showed no perceptible change even at the end of 8 days storage. Body outline was smooth, color unchanged, and the blood vessel pattern distinct. No histological study was made of the tissues previous to transplantation since the evidence in the grafts was taken as indicating the capacity of survival and the viability of the embryonic material. In all the experiments large, reddish, well-vascularized grafts were secured. Cyst formation was more common in transplants from the embryos which were preserved longest. These were also progressively larger and filled with fluid. In sections they were generally lined with gut epithelium. Larger and more numerous grafts were secured from transplants of material up to 2 to 3 days of isolation, while after this time the number and size decrease.

A. After storage for 1 to 3 days

1. *Ectodermal derivatives.* Nervous tissue grafts of bits of cerebral hemisphere and cerebellum together with small pieces of spinal cord may exhibit some evidence of disorganization and degenerative changes after a preliminary storage for 24 hours and refrigeration at 5°C. The tissue is recognizable but there is often blood extravasation, phagocytosis, and leucocyte infiltration, which may result in disturbance of form and regional organization. It is well known that the nervous system is one of the first to show effects of vascular disturbance. While the tissue may survive more than 2 or 3 days exposure with little degenerative evidence, the amount is often scarce and fewer transplants are successful (fig. 8). Nervous tissue often lines cyst-like cavities filled with fluid which coagulates in the fixing reagents. Epidermis is one of the most resistant of the tissues studied. Three days exposure of the donor makes practically no change in the graft picture. Hair development is also typical and abundant.

Several grafts of the ear were obtained. In one of a 13-day rudiment the differentiation is quite comparable to that of fresh transplants even though the donor was stored for 24 hours. Both the eye and ear showed advancement over transplants of brain or spinal cord. In this particular graft the epithelium is flattened throughout, two sensory areas are present, the endolymphatic duct is recognizable and cartilage almost surrounds the labyrinth, which is $1125\ \mu$ in length.

The graft of a 15-day rudiment, the donor of which was chilled for over 2 days, is larger ($1875\ \mu$ in length) but is very distorted like that of the earlier stage. Morphogenesis has been disturbed to such an extent that it is difficult to identify parts. However the endolymphatic duct, cochlear rudiment, utriculus, one semi-circular canal and one large sensory area with a rudiment of a cupula can be identified. In another graft of the same stage two large cyst-like structures are broadly connected and from these extend various blind tubes. Identification of the parts is impossible. A graft of a 13-day rudiment was secured from a donor stored for 3 days. This graft is of the type described above but it measures only $990\ \mu$ in length. After 4 days storage only small vesicular structures are obtained.

The transplanted eye rudiment of 13- and 15-day embryos survives almost as well as does the ear. A 30-hour exposure does not appear to influence its differentiation. As is often the case, eye grafts are usually distorted due to the tensions and pressures within a graft (fig. 4); but occasionally the graft eye may be quite typical in appearance as seen in figure 3, a graft of material kept 4 days before transplantation. The sensory retina is generally folded and may be of a disproportionately large amount in comparison with the other eye parts. The position and general structure of the lens is usually typical but its cells are irregularly enlarged and often of huge size. It is the most resistant of all the eye structures and may persist when all else has disappeared. In some cases the lens is widely separated from the much convoluted retina and there is no approach to a normal relation-

ship between the various parts. Increasing evidences of degeneration and cellular invasion appear in the grafts after 3 days exposure.

2. *Endodermal derivatives.* Liver tissue is one of the more sensitive of the embryonic rudiments tested. Even a 1-day isolation may provoke degenerative effects. The cords of the developing liver are generally rather close together but may be separated by sinusoidal spaces filled with non-nucleated blood cells and by unorganized darkly staining connective tissue. Cell outlines of the hepatic cells are often uncertain. Two days exposure results in much degeneration although small masses may still be found occasionally. Pancreas is also seen in grafts of 1-day isolation. It is always very retarded in development, small in amount, and the branching cords are often widely separated by connective tissue. In some parts the cords have a lumen though in others it is lacking. No islet tissue was seen.

Transplants of parts of the digestive and respiratory tracts survive many days exposure. For example in grafts of 13- and 15-day embryonic material from donors exposed for 3 to 5 days, the oesophagus, stomach and intestine (fig. 5) differentiate in a typical fashion. Transplants always form closed cysts containing fluid which seems to increase in amount with age. In the intestinal grafts no villi are found and usually there is little folding of the epithelium. This may be due partially to the distension by accumulated fluid of the cyst-like vesicles. The respiratory system, lung, bronchioles and trachea differentiate in a similar way to fresh tissue (figs. 1 and 7).

3. *Mesodermal derivatives.* Parts of the mesonephric kidney do not always survive transplantation as the percentage of takes is low. Differentiation is typical but retarded and often disorganized in grafts of material transplanted after 48 hours exposure of the donor. Regional parts of the uriniferous tubules are recognizable, i.e., secreting, collecting and renal corpuscles with glomeruli. Graft H 1b (fig. 2) may be taken as an example. It is a rather large graft

in the form of an elongated or finger-like mass and shows considerable darkly-staining tissue between the tubules. There is no capsule. The amount of intertubular tissue is much larger than normal and probably represents some tissue invasion. In most cases the glomerular component of the renal corpuscle is shrunken. A bit of wolffian duct is present at one side of the graft and several collecting tubules open into it. The wall of the duct consists of two muscle layers while the lumen is filled with large, lightly staining vacuolated cells. Grafts of the 15-day organ are larger and often show wolffian duct, medullary and cortical portions and frequently a lobed appearance. After 3 days exposure, the kidney tissue shows some effect. In one graft, only a few scattered tubules survive along with an occasional small renal corpuscle and a bit of wolffian duct. In other grafts the results vary.

Cartilage and bone grow and differentiate well even after several days following storage of the donor embryos. The cartilage is typical in appearance, enclosed by a perichondrial sheath, and usually is in the form of rounded or elongated masses. Its replacement by perichondrial and endochondrial bone is typical. Membrane bone differentiates normally in grafts (fig. 6), as well as skeletal muscle and developing teeth.

B. After storage for 4 days and longer

After 4 to 5 days exposure the resulting grafts are all quite small, dark red in color, and fewer in number. Histological examination reveals the fact that much of the transplanted material has failed to survive. However there is found typically appearing oesophagus, intestine with epithelial folds and muscle coats, stomach, unidentifiable glandular tissue in the form of branching cords, trachea and lung tissue, cartilage enclosed by perichondrium, lens of the eye showing anterior epithelium and nucleus of lens fibers, very small cystic inner ear partially enclosed by cartilage, epidermis, hair papillae, membrane bone, skeletal muscle, and also various epithelial tubes and cellular aggregations.

Figures 3 to 8 are illustrations of some of the surviving material.

The survivors represent derivatives from all three germ layers. Certain mesodermal derivatives such as the kidney, along with endodermal structures such as pancreas and liver, do not survive. Occasionally an eye survives (figs. 3 and 4). The lens is the most resistant of the eye structures, and in extreme cases is the only part to persist. A few small remnants of tissue of the central nervous system are found but they are disorganized and abundantly infiltrated with foreign cells. However this is not always the case as in one graft, brain tissue was seen in a good state of survival as the wall of a large flattened cyst (fig. 8).

Grafts of embryonic tissues from embryos stored for 8 and 9 days show markedly the effect of isolation of the embryo from any vascular supply and its exposure for such a long time to a very low temperature. It is quite probable, on the basis of the shorter storage periods, that the low temperature is not as harmful as the severance of the blood supply. Epidermis, hair, cartilage, a little bone, skeletal muscle, lens of the eye and occasionally some intestine, and lung appear in the grafts. The grafts are even smaller as well as the respective tissue masses contained in them. The number and amount of survival represents but a small part of the amount of material transplanted. A longer storage period sees the disappearance of all rudiments except epidermis, hair, cartilage and bone. This result is comparable to that secured from heteroplastic transplants of fresh embryonic rat, mouse and rabbit tissues to the adult.

DISCUSSION

Evidence is gradually accumulating to show that mammalian embryonic tissues will survive exposure for short periods, at least, to subnormal temperatures. One of the earliest observations to indicate this was that the exposure of the rabbit embryo or parts of it to room temperature during the period necessary for isolation, or between removal

from the uterus and transplantation, did not provoke harmful effects. Later it was found that primitive streak to early somite stages in utero will survive a temperature of 6°C. for about 30 hours at least (Waddington and Waterman, '33). No development occurred during this period. The blastocyst stage in utero has also been kept over night (Waterman) without apparent injury except through collapse of the vesicle due to loss of fluid.

In the present study older embryonic material has been tested as well as the time limit of survival. From the results secured from material stored for 1 to 3 days, with certain exceptions such as kidney, liver, pancreas, nervous tissue, it would appear that most of the embryonic tissues and rudiments tested were not harmed to any appreciable extent. Cell conditions point to healthy differentiating grafts, comparable to those of fresh tissue, which could have survived for a longer period. Thus embryonic tissue is quite resistant to the conditions of the experiment. The interruption of development has provoked no change and it is resumed where it left off. Furthermore the wide variety of the surviving tissues points to no particular difference in the survival ability of the tissues or rudiments from any one germ layer. The results secured from transplants of the 13-day embryo did not differ from those of the 15-day stage.

Grafts of 8-day material contained epidermis, hair, cartilage, skeletal muscle, bone, lens, some trachea, and occasionally intestine and lung. A longer period of exposure permits the survival of chiefly epidermis, hair, cartilage and bone, which fact compares closely with the results of the heteroplastic transplantation of fresh tissue of rabbit, mouse and rat embryos. It is concluded from these results that rabbit embryonic tissues may be successfully maintained without fatal injury outside the uterus for several days at least and then used in explanation and transplantation studies.

Others have also found that early embryonic tissues of warm blooded animals will survive subnormal temperatures. Nicholas ('34) studied the survival of rat embryos in different physiological solutions and at different temperatures.

They withstood 6 hours at 37.5°C. without signs of histolysis, and 12 hours at 24°C. after which histolytic changes were visible. After 72 hours at 24°C. the embryo maintained an apparently normal form but cellular structure was atypical and degenerative changes were visible. In no case did he find that any differentiation had occurred during the experimental period. From this he concludes that subnormal temperatures are not an immediate factor in causing embryonic death (see also Alvarez, '37). In another experiment the pregnant uterus was placed in a subcutaneous position but development was normal.

Nicholas ('38) has also studied the development of rat embryos in a circulating medium. Temperature variations within rather wide limits seemed to have slight effect upon comparable stages. Gradations of 5° ranging from 70°F. to 110°F. were employed. Thus the temperature range during which the rat embryo may exist is much wider than would be imagined. Temperatures below 80°F. affected the heart beat.

The survival of tissues from 6- to 12-day chick embryos kept in Ringer's solution at 5° and 10°C. has been studied by Bucciante ('31). Growth in vitro was the test of viability. Tissues varied in survival time, over 21 days for skin to 3 days for the blood elements in spleen and for hepatic cells. Ringer's solution appeared to wash out substances necessary for growth. Cooling down to 0°C. lengthened the survival time but temperatures below 0°C. shortened this time. According to Shipp ('37) embryo juice may be obtained from 8-day chick embryos which have been stored at 10°F. for over 6 months, and a greater growth is secured than from fresh embryos.

SUMMARY

By the method of omental transplantation the survival of 13- to 15-day rabbit embryonic tissues has been tested following removal from the uterus and stored at a temperature of 6°C. With the exception of liver, pancreas, kidney and nervous tissue, 2 to 3 days storage has little effect upon the quality and quantity of the differentiations obtained. Many tissues and organ rudiments survive even 8 days exposure while a longer period finds chiefly epidermis, hair, bone and cartilage appearing in the grafts. The size becomes appreciably smaller with longer exposure. Subnormal temperature is apparently not alone an immediate cause of tissue death (Nicholas). It would appear therefore that certain rabbit embryonic tissues may be stored for several days at least and still be employed successfully in transplantation studies.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

(All figures are photomicrographs, $\times 130$)

1 Section of a graft of the trachea (H2b3), showing epithelium and cartilage nodules after 10 days growth in the adult omentum. Donor embryo was stored in a refrigerator at 5°C. for 2 days previous to transplantation.

2 Section through kidney graft (H2b12) from material stored for 2 days. Secreting tubules and malpighian bodies are visible. The glomeruli are shrunken.

3 Section of an eye graft (H4a) from embryos stored 4 days. Lens and sensory retina are visible but the tapetal layer lacks pigment. This is the nearest approach to a typical eye structure obtained.

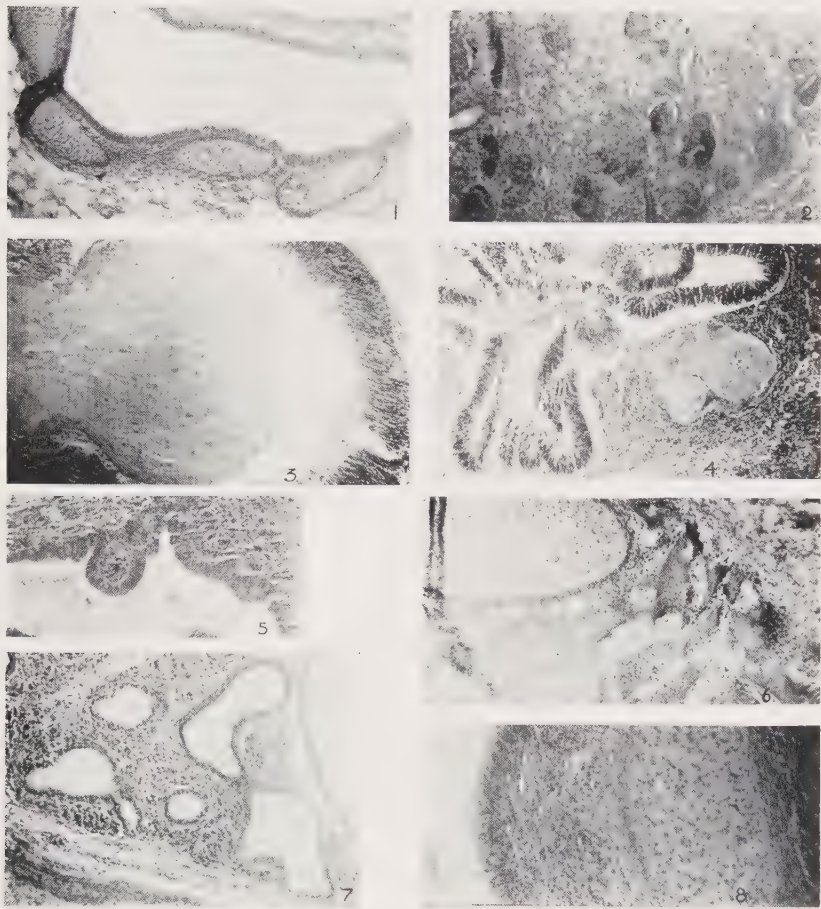
4 Section of another eye graft from the same embryos as figure 3. This is a 10-day omental graft of a 13-day rudiment, total age is 27 days. The large amount of convoluted retina lies to the left of the irregularly shaped lens.

5 Portion of a cross section of an intestinal cyst. Previous to transplantation the donor embryo was kept at 5°C. for 5 days. The transplant has formed a large cyst-like body lined with intestinal epithelium.

6 Section of a graft of a small part of the pharyngeal region of a 13-day embryo stored for 5 days. Hyaline cartilage is visible in the upper left of the photograph and developing membrane bone in the lower right.

7 Portion of a graft of developing lung from the same embryos as in figure 6.

8 Hyaline cartilage and nervous tissue (left) in a graft of the same embryonic material as figure 6.



THE PRODUCTION OF PIGMENT IN GRAFTS OF MOUSE SKIN GROWN ON THE CHORIO- ALLANTOIS OF WHITE LEGHORN CHICKS ¹

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ONE PLATE (FOUR FIGURES)

The extensive work of Hiraiwa ('27) and of Nicholas and Rudnick ('33) has provided a description of the ectodermal derivatives in chorio-allantoic grafts of whole and of partial rat embryos. They found that although the differentiation of the derivatives of each germ layer is extremely variable there was always progressive differentiation at stages preceding the twelfth day. In the 12-day grafts, however, Nicholas and Rudnick state that the rule is regression and degeneration of the graft.

The purposes of this paper are to study 1) the fate of mouse skin when placed on the chorio-allantoic membrane of chicks, 2) whether the inhibitor of pigmentation present in the White Leghorn host has any effect upon pigment formation in the graft of mouse tissue and 3) the behavior of these grafts when replaced on a mouse as the second host.

White Leghorn eggs were procured from the Macdonald College farm. Eggs containing 9-day embryos were placed on end and a day later a circular section of shell removed. The piece of mouse skin was placed on the chorio-allantoic membrane, dermis on the membrane, the egg shell replaced and sealed with a vaseline compound. The chick was then allowed to continue its incubation until the grafts were removed in from 3 to 9 days.

¹ Research supported by a grant for genetics and cytology to McGill University from the Rockefeller Foundation.

It was not considered necessary to work with mouse embryos of less than 15 days of age as this stage is much previous to the time of formation of hair follicles and pigment. Furthermore, the work of Hiraiwa and of Nicholas and Rudnick, though with rats, is adequate for a general conception of skin development in mouse embryos of 12 days or less. Our work on mice was concerned with stages between the fifteenth day of embryonic life and the third postnatal day. This covers a period antedating visible evidence of hair follicle formation and lasting until after the eruption of the hair from the skin.

EXPERIMENTAL

1. The fate of mouse skin on the chorio-allantois

Whereas Nicholas and Rudnick transplanted whole or partial embryos, the present work deals only with grafts of skin and such underlying tissues as may have been included by accident. The degree of incorporation of the grafts was variable; sometimes the donor tissue was in contact with the host mesenchyme over only a small area. Differentiation of the skin and hair follicles was never quite normal as orientation of the hair follicles was entirely at random within the graft.

Great care was exercised in placing the skin flat on the chick membrane but within 48 hours the graft always assumed a somewhat globular form, the free edges of the skin having grown together to a large extent. The less successful grafts become completely infiltrated with large sinuses and vessels filled with chick blood. Providing that hatching does not intervene, this infiltration of vessels will lead to the eventual destruction of the graft.

The skin of the youngest embryo used (10 mm., about 15 days) had not differentiated its fundamental layers; the cells were not arranged in the orderly formation characteristic of the stratum germinativum (malpighian layer). This graft (1296) remained upon the chick membrane for 7 days and parts of it were still in good condition as evidenced by the

plentiful mitoses. The skin continued its differentiation, forming the easily recognizable stratum germinativum. This layer of large cells, which are arranged evenly side by side, seems to be oriented without regard to the surface of the graft, hence if hair follicles are to arise from it they will likewise have only a random relationship to the surface of the transplant. One large hair follicle which contained a hair was produced, as well as several rudimentary hair follicles. Only a small amount of pigment was formed, but more would not be expected in tissue with a total age of only 22 days (15 days embryonic plus 7 as a graft). It is clear, however, that it is possible for undifferentiated skin from a 10-mm. embryo to differentiate and produce hairs when placed upon the chick chorio-allantoic membrane.

Sections of grafts of skin from 12-mm. and 18-mm. embryos showed no distinct hair follicles, probably because of their destruction by blood vessel invasion during the 9 days the grafts were left on the chick membranes. These grafts had few mitoses and were in poor condition when recovered.

The healthiest grafts of the experiment were of skin from a 19-mm. embryo (1254). The transplants remained on the host for 8 days and continued active until removed for sectioning. Figure 1 is a section through the skin of the embryo at the developmental stage when the grafts were placed upon the membrane. The thin uniform layer of cells of light staining properties is the stratum germinativum which differentiated but which has not yet commenced the differentiation necessary for the production of hair follicles. Figure 2 shows a section through one of the recovered grafts and demonstrates how a hair follicle will develop without the usual relationship to the stratum germinativum and to the surface of the embryo. The hair follicle of figure 2 was photographed at a higher magnification to show (in fig. 3) the completeness of development of the follicle and its contained hair and pigment. The other grafts from this 19-mm. embryo produced similar hair follicles with hairs of varying degrees of development. It may be stated then, that

grafts from 19-mm. embryos in which the stratum germinativum has differentiated will form complete hairs and pigment by self-differentiation.

Skin from a mouse 6 hours old (postnatal) was allowed to remain on the chorio-allantois for 4 days. Hair follicles, but not hairs, had formed in the donor at the time of transplantation. All the recovered grafts showed degeneration of the tissue and hair follicles. Probably the failure of the hairs to erupt was due to poor nutrition and inadequate growth of the grafts. Mitoses were lacking.

The oldest grafts were from a 2- to 3-day-old donor (1371). In the skin of the donor the hairs were completely formed and had penetrated the surface of the skin but had not emerged above it to any extent. Grafts which had remained on chicks for 3 days showed that the hairs had continued to emerge and increase in length. The hairs were abnormal presumably because of poor nutrition. The diameter of the hair was always too small for the length, which resulted in a very thin hair without proper medulla or cortex. Grafts from the same donor which were left on the eggs for 6 days showed little if any increase in size of the hairs over that of 3-day grafts while in addition there was marked degeneration.

It is possible to conclude from these experiments that grafts of embryonic skin are capable of independent self-differentiation when placed upon the chorio-allantois. With good nutritional and mechanical conditions it is expected that undifferentiated ectoderm of 12-day mouse embryos would be able to carry its differentiation practically to a conclusion with the production of emerged hairs with normal pigmentation.

Hiraiwa ('27) reported fairly complete differentiation (hair formation) from 3-mm. rat ectoderm which is much younger than the mouse ectoderm used in these experiments. The work with mice confirms the thesis that ectoderm has remarkable powers of self-differentiation and that, allowing some error for the age of Hiraiwa's embryos, his observations in regard to hair formation were probably correct.

The well-developed hairs he found in grafts of anterior thirds were probably vibrissae which develop early but the hairs found in grafts of middle thirds were probably ordinary pelage hairs.

2. The pigment inhibitor of the White Leghorn host and pigment formation in mouse grafts

White Leghorn fowl possess the gene which when present as a double dominant prevents any formation of pigment in the feathers. This inhibitor must be produced during the embryonic development of the chick and should it circulate freely in the blood stream it might inhibit production of pigment in mouse grafts. Such was not the case, however, as is well illustrated by the graft shown in figure 4. This graft came from donor 1254, the skin of which was in the developmental stage shown in figure 1. The hair follicles, hairs and pigment of the graft developed from the stratum germinativum while on the chick membrane. Three such follicles with an abundance of pigment are shown particularly well in figure 4; the slide was unstained except for a light tint of eosin. In order to show anything other than pigment in the print it was necessary to photograph with color filters (Wratten G and H) which causes the cells, other than the pigment, to appear much clearer and darker than they actually are.

It is, then, hardly necessary to state that pigment is formed normally in grafts of mouse skin on White Leghorn membranes. Apparently the White Leghorn pigment inhibitor is either not present in the chorio-allantoic blood stream, or if it is present it has no effect upon mouse pigment formation; that is, it is specific to fowls. Furthermore, there is evidence in the literature that the inhibitor is specific to White Leghorn cells in distinction to cells from fowls of other breeds.

Willier, Rawles and Hadorn ('37) transplanted colored ectoderm to White Leghorns; both the hosts and donors were 75-hour embryos. At this early stage no feather germs have been formed. The colored grafts developed fully pigmented

feathers on the White Leghorn hosts, the inhibitor of the host having no effect on the donor tissue. Apparently the inhibitor takes effect only in White Leghorn cells.

Of particular interest in relation to the inhibitor in White Leghorns is the work of Dorris ('38). She grew neural crest tissue from early somite stages in plasma-extract medium in depression slides for periods ranging from 7 to 15 days.

Explants from embryos of pigmented breeds, such as the Black Minorca, Australorp and Barred Plymouth Rock, are made up largely of amoeboid cells filled with refractile granules which gradually acquire pigment reaching full intensity in many cases by the third day in vitro. No pigment cells were obtained from neural crest of Rhode Island Reds or White Plymouth Rocks (recessive white), although cultures of White Leghorn (dominant white) having a color factor plus inhibitor produced them almost invariably. When inserted into the limb bud of a 3-day host, neural crest from Australorp (dominant black) produces melanophores in the dermis and in the feather germs of both White Leghorn and White Plymouth Rock hosts, while none, or very little, pigment resulted from grafts to Rhode Island Red embryos.

It is remarkable that White Leghorn cells in tissue culture produce pigment, and it is a possible answer to the results of Willier, Rawles, and Hadorn ('37) in which 75-hour-old White Leghorn ectoderm seems to have produced colored plumage instead of white when placed on colored hosts. Danforth and Foster ('29) used much older material (newly hatched) and found that the grafts of White Leghorn skin remained white throughout the life of the colored host.

Thus it seems that young ectoderm (75 hours or less) produces pigment in tissue culture and grafts but by hatching time (or by the time the feather germs are formed) the inhibitor is present and thereafter white feathers will be produced irrespective of the host. It should be possible, then, by appropriate operations to determine at what time in development, between 75 hours and appearance of the feather germs, the inhibitor becomes effective. Presumably the inhibitor is somewhat 'weak' in its expression as it is well

known that White Leghorn fowls heterozygous (but not those homozygous) for the inhibitor gene produce many colored feathers. Removal of White Leghorn ectoderm before the inhibitor acts, and subsequent growth in tissue culture, or on some other breed of fowl, is probably sufficient to prevent action of the inhibitor at the critical time. It is hoped that chick embryologists will carry on experiments which will confirm or disprove the above hypotheses. This problem of the White Leghorn inhibitor is directly related to our efforts to discover how and at what time pigmentation genes cause their effects in mice.

3. Behavior of chorio-allantoic grafts of mouse tissue when placed upon mice as the second host

Most of the grafts of mouse tissue were removed from the chorio-allantois and then placed on newly born mice as the second host. This would allow observation of any change in pigmentation which might result from the exposure to the chick allantois. The tissues were removed from embryos of various lengths, left on the chick membranes for 48 hours or more and then placed on back or belly of a newly born mouse. Dorsal tissue was always placed on the belly of the mouse, and ventral on its back thus allowing certain identification later on, as described by Reed and Sander ('37).

The grafts did poorly on the mice, probably because the pieces of mouse tissue were usually surrounded by chorio-allantoic tissue which may have functioned as an insulator and prevented incorporation of the graft. Two grafts from a 21-mm. embryo (1604) were successful and produced good growths of hair. As the hair was in both cases of the color expected, the exposure of 48 hours on the chorio-allantois was apparently without effect. A highly successful method of placing mouse tissue on agar plates for several days' treatment with various chemicals and then growing the skin on newly born mice was devised at this stage of our experiments; consequently, attempts to achieve results with the

chorio-allantoic grafts were discontinued. The agar plate experiments will be reported elsewhere.

SUMMARY

A study was made of the differentiation of embryonic mouse skin on the chorio-allantoic membrane of White Leghorn chicks. The undifferentiated skin from 10-mm. embryos produced a stratum germinativum which then differentiated to produce hair follicles, young hairs and some pigment. Nineteen-millimeter embryos provided grafts which formed healthy hair with normal pigmentation. It is probable that undifferentiated ectoderm from 10-mm. embryos would be able by self-differentiation to produce mature emerged hairs and normal pigment under proper nutritional condition.

It was found, by use of chorio-allantoic grafts, that the White Leghorn host is not capable of inhibiting the formation of mouse pigments, nor does the chick host affect the pigmentation of the mouse hairs when the graft is allowed to grow upon mice as the second host. This shows either that the White Leghorn pigment inhibitor is not present in the chorio-allantoic blood stream, or that if it is present it has no effect upon mouse pigment formation.

It has been known for some time that the inhibitor of White Leghorns has no effect on grafts from colored breeds of fowls and recently Dorris ('38) has shown that neural crest cells from young White Leghorn embryos produce pigment in tissue culture and in grafts to embryos of other breeds. We suggest that by appropriate operations the time at which the inhibitor becomes effective in White Leghorns could be found.

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PLATE 1

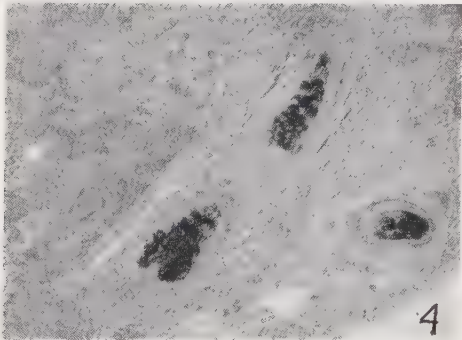
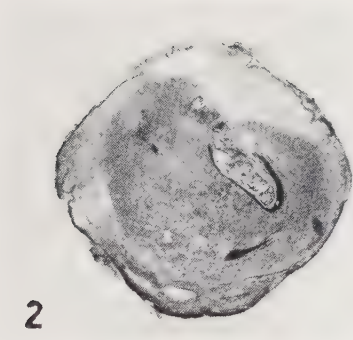
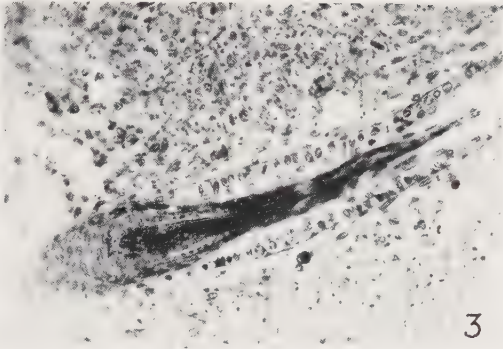
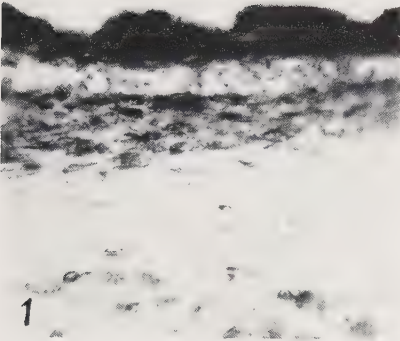
EXPLANATION OF FIGURES

1 Skin and underlying tissue of the 19-mm. embryo (1254). The thin uniform layer of cells with light staining properties is the stratum germinativum. Later in development hair follicles arise from cells of the stratum germinativum. $\times 840$.

2 Section through a chorio-allantoic graft of skin from embryo 1254. Irregular differentiation occurred with the production of scattered hair follicles. One of the follicles is shown in the lower right. $\times 50$.

3 Enlargement of the hair follicle shown in figure 2. $\times 560$.

4 Three hair follicles in the chorio-allantoic graft from embryo 1254. The pigment granules show particularly well in this preparation which was lightly stained with eosin. $\times 560$.



UNUSUAL REACTIONS OF GASTRIC MUCOSA FOLLOWING HYPOPHYSECTOMY

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ONE FIGURE

During the past year the writer has made a histological study of some of the tissues, other than the endocrine glands, of dogs from which the pituitary gland has been removed. One of these hybrid groups showed striking changes in the gastric mucosa. This was a group of litter mate F_3 Dane-St. Bernard pups from Dr. C. R. Stockard's hybridization experiments that had been hypophysectomized when they were approximately 2 months old. All the dogs recovered from the operation promptly, but shortly after, all of them died. The shortest post-operative survival time was a little less than 3 days while the longest was 16 days. At autopsy the stomach showed many small yellowish-white specks in the gastric mucosa, more numerous toward the cardiac end.

The important fact is that although a large number of dogs from various breeds have been hypophysectomized, the post-operative mortality has been low; only these F_3 Dane-St. Bernard pups have shown 100% mortality and have exhibited the condition of the gastric mucosa here described.

Microscopic sections from the cardiac region of the stomach showed great abundance of very small lymph nodules, the lymphoid tissue increasing with the length of survival. Thus in dog 2517, which lived less than 3 days after operation, there were several well-defined lymph nodules between the muscularis mucosa and the base of the gastric glands. Except for the slight increase in the lymphatic tissue the stomach appeared normal. In dog 2516, which died 9 days after

operation, there was a great increase in lymphatic tissue just beneath the gastric glands. In many places this lymphatic tissue pushed the gastric glands aside, or replaced them, coming to lie beneath a single layer of epithelial cells lining the cavity of the stomach. These lymphatic areas undoubtedly explain the whitish specks seen in the stomach lining at autopsy. In dog 2514, which died 16 days after hypophysectomy, the gastric mucosa was even more highly modified. There was not only a tremendous amount of

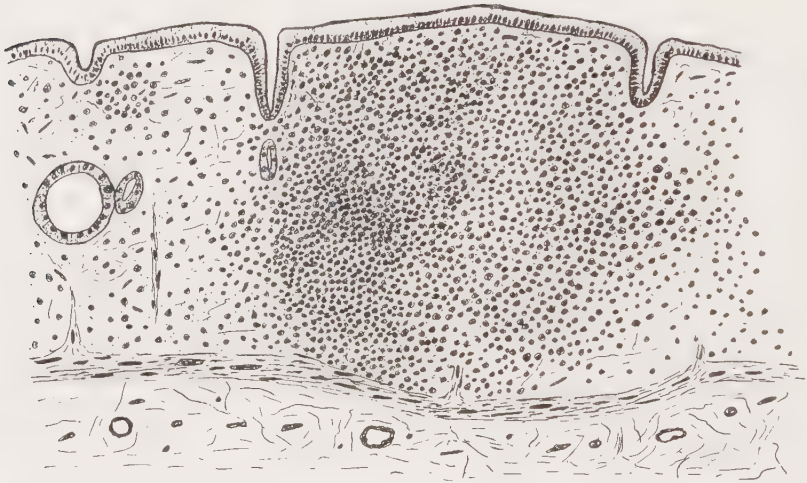


Fig.1 A diagrammatic drawing of a portion of gastric mucosa showing the position of abundant lymphatic tissue and absence of gastric glands. Only a small part of the submucosa is represented.

lymphatic tissue present but the tissue was changing as evidenced by the presence of many stellate cells resembling primitive connective tissue. The number of gastric glands was reduced and there were areas in which the epithelium had been smoothed out obliterating the gastric glands. Not even shallow pits remained. This is apparently due to abnormal stretching of the surface by the rapid infiltration of the lymphatic tissue. Occasional small cysts, lined by low cuboidal epithelium, were seen in the mucosa. These may

have been the enlarged bodies of occluded glands. There also appeared to be a marked reduction in the number of parietal or acid-forming cells; however, this may be questioned on the ground that all the samples of tissue from different stomachs did not come from comparable regions.

There were also striking histological changes in the liver of these pups following the operation. Varying degrees of congestion were found in which the hepatic cords were unusually well marked, and there was apparent depletion of glycogen. An abnormally large number of polymorphonuclear leucocytes were present in the hepatic sinusoids of all specimens. In dog 2516 this was particularly obvious, and the condition might be due to drainage of leucocytes from the alimentary wall through the portal system into the liver. Marked histological changes also occurred in the livers of several other hybrids following the removal of the pituitary.

After hypophysectomy, one of the outstanding reactions noted upon recovery was a marked change in the desire of the dogs for certain types of food. The modifications in the stomach and liver are no doubt involved in this change in choice of food.

It is a pleasure to acknowledge the help of Dr. W. T. James of Cornell University Medical College, with whose cooperation this work was accomplished.

TESTICULAR FUNCTION AND THE ACTION OF GONADOTROPIC AND MALE HORMONES IN HYPERTHYROID MALE RATS ¹

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Relatively few attempts have been made to study testicular function in hyperthyroidism. Monterosso ('12), Gudernatsch ('15) and Belawenetz ('28) found that thyroid fed rats suffered decreased testicular weight. Courier ('21) discovered no change in testicular structure of thyroid fed cats, and later ('28) reported that thyroid feeding of rats and dogs caused precocious puberty. In contrast, Da Costa and Carlson ('33) found thyroid feeding slightly delayed puberty and decreased the testicular weight in rats. Hoskins ('16) and Herring ('17) working with rats, Cameron and Carmichael ('20) with rats and rabbits, all report slightly heavier testes as a result of thyroid feeding. Cohen ('35) feeding relatively large amounts of thyroid found testicular weight increased and slight decrease in seminal vesicle and prostate weight. The structures were normal histologically.

Obviously, knowledge of the thyroid-testis relation is exceedingly limited, for even the conclusion that relatively high doses of thyroid substance damage the testis is unwarranted by evidence found in the literature. Experiments were planned to clarify the functional relation of the thyroid and testis. It was proposed first to demonstrate the effect, if any, of administration of desiccated thyroid and thyroxin on the germinal and endocrine activity of the testis; second, to determine the action of gonadotropic and male hormone in

¹ Aided by a grant administered by Dr. P. E. Smith from the Rockefeller Foundation, New York City.

hyperthyroid animals; and third, to ascertain the effect of thyroxin injection on the gonadotropic hormone content of the blood.

EXPERIMENTAL

The earlier work was done on rats weighing from 200 to 300 gm., the later and major portion on litters of Long-Evans strain of known age. The mice were of highly inbred Bagg albino stock. Na-thyroxin, product of the British Drughouse, and Thyroxine crystalline synthetic, made by Hoffman-La Roche et Cie, were used.²

Testicular endocrine activity was judged either by the weight of the entire reproductive tract, exclusive of the testis and epididymis, or by the seminal vesicle and prostate cytology test. This latter test is best adapted to conditions where very little androgenic hormone is present, whereas weight of the entire tract is best adapted to show gross variation from the normal level in the amount of testis hormone available.

Spermatogenetic activity was determined by counting the sperm in the tail of the epididymis. The epididymis was weighed, the tail minced, and the fragments thoroughly washed with several changes of saline to remove the sperm so that an accurate count could be made on an haemocytometer. It was found most satisfactory to suspend the contents of one epididymis in about 400 cc. of saline. Ten counts made of each sample were found adequate, for ten additional counts did not affect the average values obtained.

The great variation in number of sperm found in normal animals, even among litter mates, made it necessary as a control measure to count the sperm present in one epididymis before beginning treatment. Following the treatment a count

² The pregnancy urine extract used was Antuitrin S and the pituitary gonadotropic material was an alkaline extract of dried sheep pituitaries, both furnished by Parke Davis and Co. The sheep pituitary powder was extracted for 24 hours in the cold with dilute NaOH, centrifuged, and the supernatant fluid neutralized with dilute HCl and recentrifuged.

Prof. F. C. Koch and Dr. T. F. Gallagher prepared the male hormone from urine and reported that there were 25 B.U. per cubic centimeter of olive oil.

was made on the remaining epididymis at autopsy. Normally the number of sperm in the right and left epididymis is approximately the same, but following unilateral castration the number in the remaining epididymis is increased (Smelser, '33). This increase was found to be consistent and the values obtained were statistically significant. The effect of the experimental treatment on spermatogenesis is shown by its influence on the expected increase in sperm following unilateral castration.

1. Effect of thyroxin injection

Twenty-five adult male rats, divided into several groups, were unilaterally castrated and sperm counts made, ten uninjected unilateral castrates serving as controls. For 30 days the animals were injected daily with 0.028 to 0.28 mg. Na-thyroxin. The higher dosages were decidedly toxic (three of the five animals receiving 0.28 mg. died) while the lowest amount caused little or no weight loss.

The testis weight of each animal receiving the lowest dosage (0.028 mg.) showed a slight increase over the weight of the control testis, as did the testis weight of the uninjected controls. However, testicular weight of all groups subjected to higher dosages decreased below that of the control testis.

Histological examination of these testes in no case revealed a striking deviation from normal, even the testes of animals treated with the largest amount of thyroxin containing many active seminiferous tubules. None showed degenerative changes in any way comparable in animals subjected to x-ray, or made cryptorchid. Fewer tubules contained mature sperm than in the controls, and in many instances the tubules were smaller in diameter. These changes, although difficult to estimate quantitatively, were sufficient to account for the decrease in number of sperm discussed later.

The seminal vesicles and prostates of this series were markedly affected by all dosages. Injection of the larger amounts of thyroxin produced pronounced atrophy of the accessories. The seminal vesicles were small, flabby and, in

those animals receiving more than 0.056 mg. Na-thyroxin daily, completely devoid of secretion. Accessories of those rats receiving less than 0.056 mg. daily in no case approached normal, although a small amount of thin, watery fluid was present in a number of instances. In several cases the accessories, examined after 2 weeks treatment, were found well on the way to the atrophic condition described above.

TABLE 1

The action of Antuitrin S in correcting the disabilities caused by thyroxin administration

LITTER NO.	THYROXIN INJECTED, 0.01-0.02 MG. PER 50 GM. BODY WEIGHT DAILY				THYROXIN AND ANTUITRIN S (25 R.U. DAILY)				THY-ROXIN DOSE	NUM-BER OF DAYS
	Access-ory weight	Testis weight	Sperm number	Body weight	Access-ory weight	Testis weight	Sperm number	Body weight		
	gm.	%	%	%	gm.	%	%	%	mg.	
1	1.390	+7	-42	-31	3.768	-6	-22	-22	0.01	46
					3.023	-6	+12	-22	0.02	34
2	1.772	0	-42	-33	5.097	+6	+128	-16	0.02	34
3	2.506	+6	-16	-10	4.039	+7	+70	0	0.01	47
	2.263	+3	-28	-7		...		...	0.02	55
4	2.565	+2	-7	-18	4.750	+18	+30	-16	0.02	34
	2.393	+1	-34	-10	2.904	-4	-13	-35	0.01	34
5	2.795	+16	+24	0	6.237	+4	+53	+7	0.01	47
6	2.105	-3	-36	-16	2.502	-1	-5	-19	0.02	31
					2.888	+4	0	-20	0.02	31
Av.	2.223	+4	-22.6	-15.6	3.910	+2.4	+28	-15.9		

Testis weight and sperm number are expressed in per cent of the values obtained by unilateral castration at the beginning of the experiment.

In a second series of experiments, weights of the accessories yielded a more objective measure of the effect of thyroxin injections, weights in the hyperthyroid animals averaging 2.2 gm. compared to about 3.5 gm. in normal animals (table 1).

In all but the lowest dosage group the seminal vesicles were essentially castrate in type. There were no 'haloed' secretion granules such as described by Moore, Price and Gallagher ('30) and the cells were cuboidal, rather than columnar, there being little or no cytoplasm distal to the nucleus. Sections

of the seminal vesicles from rats receiving the milder treatment (0.028 mg. Na-thyroxin daily) revealed a higher epithelium in three cases, presenting some, though far less than the normal amount, of granular cytoplasm.

Sperm counts were not made in the first experiments since the necessary methods had not been sufficiently perfected. However, epididymal weight in all cases, coupled with sperm counts in most cases, clearly demonstrates the marked injurious effect of hyperthyroidism on sperm production in all but the lowest dosage group. Following unilateral gonadectomy, epididymal weight as well as sperm number invariably increased in controls. Excluding the lowest dosage group, the epididymal weights were decreased by the thyroxin injections. In the lowest dosage group, epididymal weights of three of the five animals were decreased. In these experiments, some correlation between epididymal weights and sperm production was evident but weights cannot be taken to indicate sperm production alone.

Sperm counts in animals receiving minimal treatment (0.028 mg. thyroxin daily) do not indicate great damage to spermatogenic activity, confirming the data on epididymal weights. In each case the sperm count was considerably higher at the close of the experiment than at its initiation. Dosages higher than these, however, decreased spermatogenesis so that, in some cases, the epididymes were practically devoid of spermatozoa. Table 1 shows that the sperm number of the thyroxin injected animals in the second series decreased an average of 22%.

2. Recovery

The relatively slight effect of thyroxin injection on testis structure suggests that impairment of testicular function is dependent upon continued treatment. Three normal animals were treated with Na-thyroxin for 1 month, causing a 15% to 30% body weight loss. One epididymis and seminal vesicle was then removed and the injections discontinued.

The seminal vesicle histology approached that of castrates, there being no secretion granules and a cuboidal epithelium, although the testes were essentially normal in structure. Body weight was rapidly regained, and at autopsy 30 days later the reproductive tract was quite normal. The seminal vesicles were distended, and the columnar epithelial cells were filled with typical secretion droplets. The testes and epididymes were heavier, the testicular tubules active, and the number of spermatozoa was increased.

3. Effect of thyroid feeding

To check the method of administration, and to obtain a basis for comparison with other studies, the effect of feeding desiccated thyroid gland was determined. Five adult male rats were unilaterally castrated to secure reference controls on testis weight and number of sperm. For 30 days each received daily one Burroughs and Wellcome 'Tabloid,' containing the equivalent of 0.324 gm. fresh gland.

The results were comparable to those of the thyroxin series. Testicular weight was but slightly decreased, although body weight was reduced from 13% to 35%. Sections of the testes revealed many active tubules and no indication of marked damage, though the proportion of active tubules must have been affected for spermatogenesis was severely depressed and the epididymes, reduced in weight, contained fewer sperm in all cases. Sperm number decreased an average of 30% whereas the average in controls increased 80%. The accessories were reduced, and the seminal vesicles were small, flabby, and devoid of secretion. Sections of the seminal vesicles revealed a cuboidal secretory epithelium containing no secretion granules.

4. Repair with Antuitrin S

The above experiments show that injection and feeding of thyroid preparations depress the secretory activity of the accessories and the production of spermatozoa. The absence of any gross disturbance of testicular morphology, and prompt

recovery upon discontinuing injections, suggest that an insufficient supply of gonadotropic hormones may be responsible for the condition.

To determine if this was the case, nine pairs of litter mate adult male rats were unilaterally castrated, the testes and epididymes weighed, and sperm counts made. The animals were then injected daily with 0.01 to 0.02 gm. thyroxin per 50 gm. body weight from 31 to 55 days. One of each pair received in addition 25 R.U. Antuitrin S daily. Both groups lost approximately the same weight with the injections.

At autopsy, the testis, epididymis, and accessories were weighed, and the number of sperm was determined as before, and the differences between the values obtained at autopsy and the preliminary biopsy were compared.

As in the preceding experiments, the testicular weight of the rats receiving thyroxin alone did not change materially, but spermatogenesis was decreased, as clearly demonstrated by the sperm counts, for these showed a 22% average decrease in sperm number (table 1). The degree of suppression, as before, seemed roughly proportional to the decreased body weight.

The daily injection of 25 R.U. of Antuitrin S at least partially maintained normal spermatogenesis, for sperm counts in this group averaged a 28% gain. Three of the nine animals had a lower sperm count than before treatment, but each of these rated higher than their litter mate receiving thyroxin but no gonadotropic extract.

The seminal vesicles of the rats treated with thyroxin alone were small, flabby, and devoid of secretion, again confirming previous observations. Accessory weights (table 1) were greatly reduced from a normal average of 3.5 gm. to 2.2 gm. The simultaneous injection of gonadotropic hormone effectively prevented this reduction in each case, maintaining the average accessory weight at normal. The seminal vesicles of the rats receiving the combined injections were large and distended with secretion, demonstrating good secretory activity.

Apparently sufficient available gonadotropic hormones may prevent deleterious effects of thyroxin on both sperm production and the condition of the accessories.

5. Action of male hormone on hyperthyroid castrate males

Reiss and Pereny ('28) and Van Horn ('31, '33) reported that thyroid-fed castrated females require more estrin to produce vaginal cornification than do normals. This suggests, that the castration-like atrophy of the accessories in hyperthyroid male rats might be due to an increased amount of male hormone required to maintain them in normal condition.

Thirty-five adult rats (200 to 250 gm.) from eight litters were used to determine the effectiveness of male hormone in normal and hyperthyroid castrate rats. The animals were bilaterally castrated and each litter divided so that each member received either thyroxin, male hormone, thyroxin plus male hormone, or was reserved as an uninjected control. By this arrangement of litter mate controls, greater significance may be attached to differences between groups. The amount of thyroxin injected (0.1 mg. daily) was known to be sufficient to affect the accessories of a normal rat. Male hormone was administered in amounts within accurate assay range (2.5 to 5.0 B.U.) of the criterion used (Hansen, '32). Since oil was used as a vehicle for the male hormone, small amounts ($\frac{1}{10}$ to $\frac{1}{2}$ cc.) were injected to aid complete absorption. No oil was found at autopsy. All injections were subcutaneous and daily for from 8 to 20 days.

The rats were autopsied the day following the last injection, the accessory reproductive tract weighed, and the prostate fixed in Bouin. Secretory epithelium height in ten different regions in each prostate was measured with an ocular micrometer. An attempt was made to choose regions for measuring which were representative of the whole, and those in which curvature of the acinus distorted the shape of the epithelial cells were avoided.

Moore, Price and Gallagher ('30) and Hansen ('32) demonstrated that castration for a period of 4 to 5 days resulted in

disappearance of all 'light areas' in the prostate cells, though injection of small amounts of male hormone prevented this. Prostate epithelium height has been shown as a sensitive and reliable test for male hormone. Within 10 days postcastration epithelial height is markedly reduced, but injection of small amounts of male hormone prevents this. These two cytological criteria are particularly adapted to testing for the presence of small amounts of hormone such as those involved.

Table 2 shows that injection of from 2.5 to 5.0 B.U. daily of testis hormone maintains the accessory weights of castrates 21% above that of uninjected castrate litter mate controls. Litter mates receiving the same amount plus thyroxin failed to show this male hormone action, the accessory weights averaging only 2% over the uninjected controls. The injection of thyroxin, though reducing body weight, had no effect on accessory weights of castrates.

Cytological examination of the prostates failed to reveal the 'light areas' in secretory cells of the untreated castrates and those receiving thyroxin. However, they were readily demonstrable in each castrate treated with male hormone alone. Of the eleven castrates receiving both male hormone and thyroxin, 'light areas' were found in only two instances, both questionable, since they were faint and in but few acini.

The average epithelial height of the castrate rats receiving male hormone averaged 29% over their uninjected litter mate controls (table 2), whereas in those receiving the same amount of male hormone plus thyroxin it was but 10% higher.

On the basis of the three criteria used, accessory weight, presence of the 'light areas' in the secretory cells, and height of the secretory epithelium, it is clear that male hormone administered parenterally is markedly less effective in hyperthyroid rats. This inhibition of male hormone action by thyroxin offers at least a partial explanation of the atrophic reproductive organs in hyperthyroid animals described earlier. It does not, however, explain decreased spermatogenesis in such animals, which strongly suggests a pituitary gland involvement.

TABLE 2
Effect of male hormone on accessory weight and prostate epithelium of normal and hyperthyroid castrate rats

LITTER NO.	THYROXIN 0.1 MG. DAILY				THYROXIN AND MALE HORMONE				CONTROL NO INJECTION				MALE HORMONE				UNITS MALE HORMONE DAILY	INJECTION	days
	Access- sory weight		Epi- thelial height		Light areas	Access- sory weight		Epi- thelial height		Light areas	Access- sory weight		Epi- thelial height		Light areas				
	gm.	μ	gm.	μ		gm.	μ	gm.	μ		gm.	μ							
1	1.236	13.4	—	—	—	1.149	15.6	—	?	—	1.130	9.3	1.196	15.1	+	—	5.0	8	
2	0.789	12.6	—	—	—	1.016	15.3	—	?	—	1.044	13.9	1.171	19.8	+	—	5.0	11	
3	1.038	13.8	—	—	—	0.897	12.4	—	—	—	0.968	10.5	1.134	13.7	+	—	5.0	11	
4	0.984					0.877	11.2	—	—	—	0.911	11.2	1.004	12.6	+	—	5.0	8	
5						1.160	12.7	—	—	—	1.038	9.2	1.505	10.7	+	—	2.5	20	
						1.265	8.6	—	—	—			1.518	11.4	+	—	2.5	20	
6						1.311	11.1	—	—	—	1.318	10.1	1.670	12.8	+	—	2.5	20	
						1.353	9.7	—	—	—			1.467	14.8	+	—	2.5	20	
7						0.837	8.8	—	—	—	0.931	10.4	1.046	13.4	+	—	2.5	15	
						0.836	10.5	—	—	—			1.041	14.8	+	—	2.5	15	
8	1.047	9.4	—	—	—	1.170	12.4	—	—	—	1.091	10.5	1.314	13.0	+	—	2.5	14	
Av.	1.019	12.3				1.079	11.7				1.054	10.6	1.279	13.8					

6. *Action of gonadotropic hormones in immature hyperthyroid males*

Since evidence indicates that the hypophysis or its hormones, as well as the male hormone, are involved in the deleterious effects of thyroxin administration, experiments were devised to determine the effectiveness of gonadotropic hormones in immature hyperthyroid animals, and to ascertain whether thyroxin, administered in doses too small to depress growth, affects prepubertal development of the male genital system.

At autopsy, the testes and accessories were weighed, and the testes sectioned. Outline drawings of twenty tubules of each testis were made with the aid of a projection apparatus, the area of the tubule drawings being determined by tracing the outlines with a planimeter. Only true cross sections were chosen for drawing.

Sixty 15-day-old male rats from ten litters of six each were used. Each litter had one uninjected control, one rat received thyroxin, two received gonadotropic extract, and two gonadotropic extract plus thyroxin. Thyroxin in a dosage of 0.02 mg. per 10 gm. body weight was injected daily, an amount which does not affect body growth. Two and one-half to 5 rat units of Antuitrin S or an extract of from 25 to 35 mg. of sheep hypophyses were the daily dosages. All injections began on the sixteenth day of life; Antuitrin S recipients were carried from 20 to 26 days, those receiving sheep pituitary extract from 15 to 20 days. A long test period was used to allow sufficient time for the thyroxin to act. Animals were autopsied well before gonad development at puberty could complicate results.

Autopsy data show that injection of 2.5 to 5.0 R.U. Antuitrin S increased testicular weight an average of 9% over the uninjected litter mate controls. Sheep pituitary extract, however, produced a 40% increase in testicular weight (table 3).

Both gonadotropic preparations seemed ineffectual on the gonads of hyperthyroid animals, for the testes not only failed to increase in weight, but were as small or smaller than those

TABLE 3

Effect of gonadotropic hormones on the male reproductive system of normal and hyperthyroid immature rats

A. Sheep pituitary extract

LITTER NO.	PITUITARY EXTRACT AND THYROXIN			PITUITARY EXTRACT			UNINJECTED CONTROLS			THYROXIN				
	Accessory weight	Testis weight	Tubules	Accessory weight	Testis weight	Tubules	Accessory weight	Testis weight	Tubules	Accessory weight	Testis weight	Tubules	PITUITARY DOSE	THYROXIN DOSE
	mg.	mg.		mg.	mg.		mg.	mg.		mg.	mg.		mg.	mg.
1	225	267	442	352	386	690	257	357	723	...	...	...	35	0.01
				563	532	789							35	
2	244	293	649	388	496	766	220	159	441	190	232	490	35	0.01
	238	279	462										35	0.01
3	320	399	611	492	702	778	294	572	808	195	335	539	25	0.01
	266	338	510	516	716	752							25	0.01
4	348	505	843	672	480	846	359	514	1037	244	598	1017	25	0.01
	352	420	709	601	695	1026							25	0.01
	353	409	588										25	0.01
5	225	329	573	472	695	1034	251	393	860	...	...	...	25	0.01
	303	692	892	384	504	712							25	0.01
Av.	287	393	628	493	578	821	276	399	774	209	388	682		

B. Pregnancy urine extract

LITTER NO.	ANTUITRIN S AND THYROXIN			ANTUITRIN S			UNINJECTED CONTROLS			THYROXIN				
	Accessory weight	Testis weight	Tubules	Accessory weight	Testis weight	Tubules	Accessory weight	Testis weight	Tubules	Accessory weight	Testis weight	Tubules	ANTUITRIN S DOSE	THYROXIN DOSE
	mg.	mg.		mg.	mg.		mg.	mg.		mg.	mg.		R.U.	mg.
1	596	757	1191	586	1194	1518	550	1294	2065	388	1075	1217	2.5	0.01
	330	1113	1861	812	1192	1291							2.5	0.02
2	211	495	844	671	936	1534	201	612	1083	236	477	851	2.5	0.02
	368	887	1382	705	779	1320							2.5	0.02
3	235	281	600	441	715	1193	322	697	1152	221	706	863	2.5	0.02
	243	612	976	400	750	1033							2.5	0.02
4	287	284	469	1524	843	1306	330	630	1035	...	...	...	2.5	0.02
	370	539	867										2.5	0.02
	500	330	682	1370	908	1220							5.0	0.02
5	273	428	786	415	694	1017	331	835	895	182	320	545	5.0	0.02
				473	846	1240							5.0	0.02
Av.	341	563	966	740	886	1267	347	814	1222	257	644	869		

All injections were daily, the pituitary dose is in terms of dried anterior lobe powder, the thyroxin dose is per 10 gm. body weight and was increased as the animals gained. The figures for tubules are the average cross section, in square millimeters, of twenty tubules measured at $\times 200$ magnification.

of the rats receiving thyroxin alone. The absence of a response from the sheep pituitary injections, which caused a 40% increase in testis weight in the normal, demonstrates clearly the depressing effect of thyroxin on the action of gonadotropic hormones.

The administration of the above amounts of gonadotropic hormones was without effect on the cross section area of the testicular tubules, a fact which is particularly puzzling because it markedly increased testicular weight in the non-thyroxin treated animals. Possibly the weight increase from this treatment is due to an increase in the number or length of the tubules. Thyroxin, however, caused a marked and consistent decrease in the cross section area of the seminiferous tubules. Injection of sheep pituitary extract in addition to thyroxin did not modify this effect, but pregnancy urine extract partially maintained the tubule diameter in such animals. The series treated with pregnancy urine extract and thyroxin were autopsied when many seminiferous tubules contained metamorphosing spermatids, but not mature sperm. This stage is very definite and adaptable to the present purpose, for presence of mature sperm would indicate some stimulation, while absence of elongate spermatids would indicate suppression.

In no animal treated with gonadotropic extracts were tubules found in a more advanced stage of spermatogenesis than in the controls, but about half of those treated with thyroxin or thyroxin plus gonadotropic extract showed no metamorphosing spermatids in any of the tubules. This further demonstrates that thyroxin administration suppresses or delays spermatogenesis.

Injections of both types of gonadotropic preparations greatly increased accessory weight, the pregnancy urine causing 113% increase, the sheep pituitary extract 79%. Both failed to produce this increase when administered concurrently with thyroxin. However, accessory weights of gonadotropic treated hyperthyroids, though no greater than untreated controls, were heavier than in the thyroxin injected animals.

Careful examination of histological preparations was made to determine whether the development of interstitial cells differed between the hyperthyroid and normal gonadotropic hormone treated animals. Sections of control and experimental testes were studied as unknowns by two individuals and differentiated on the basis of the relative amount of interstitial cells. The amounts of hormone administered were too small, however, to produce marked interstitial cell hypertrophy, and the differences therefore were not pronounced. Observations were repeated on different days and the two individuals confirmed one another. In two-thirds of the cases the interstitial cell masses of the hyperthyroid rats were less well developed than in the normals receiving gonadotropic extract. This would indicate that hyperthyroidism interferes with the pituitary factor that affects interstitial cells as well as the one which controls the germinal epithelium.

Injection of thyroxin alone clearly inhibited the development of the reproductive tract. The testes were markedly reduced in weight (21%) below their controls, when the treatment extended 25 days, and spermatogenesis was delayed in comparison with litter mates. The decrease in diameter of the seminiferous tubules (33%) is further indication of the effect on the gametogenetic function. The accessory weights of these animals were 25% under those of normal litter mates, showing that the effects demonstrated in adults can be obtained in prepubertal animals. Prepubertal testis hormone secretion has been demonstrated, Wiesner ('34), Smelser and Clark ('36) and Price ('36).

7. Gonadotropic activity of hyperthyroid castrate male serum

The preceding experiments show that testicular depression in hyperthyroidism is due to suppression of the action of gonadotropic hormones. It seemed possible that this might be due to either the destruction of the gonadotropic hormones or to a more rapid excretion. Since attempts made to demonstrate gonadotropic hormone in the urine of normal, hyperthyroid, and castrate rats failed, the first postulate was tested.

Emery ('32) demonstrated gonadotropic hormone in the serum of castrate rats, securing positive reaction in the immature rat by injecting 19 cc. (14 to 16 cc. minimum) serum of castrate male rats. This amount of serum requires pooling the blood of four to six animals and was, therefore, impractical for the present experiment. By dividing 3 to 4 cc. serum of castrate male rats into $\frac{1}{2}$ cc. amounts injected twice daily into immature (22 days old) female mice, a positive ovarian, uterine, and vaginal response was obtained. This amount is obtainable from a single adult. Thus it was possible to determine the amount of gonadotropic hormone available at a given time.

Two series of experiments were performed to determine the influence of thyroxin injection on the amount of gonadotropic hormone in the blood of castrate male rats. In the first, seven litters comprising eighteen animals were used, of which six were reserved as controls. Injections of 0.1 mg. thyroxin daily were started at the time of castration and continued until autopsy, approximately 45 days later. Weight loss of the hyperthyroid castrate was marked and comparable to that of the normal hyperthyroid animals in the first experiment. In all but two instances, experimental and control sera were from litter mates, and were tested on litter mate mice (table 4A).

In the second series, thyroxin injections of 0.1 mg. daily were started 60 to 75 days postcastration and continued for 20 to 30 days. At autopsy the rats were bled and the serum assayed as before. The effect of these injections on the ovarian and uterine weight and the vaginal orifice of the test mice is given in table 4B.

It will be noted that injection of normal and hyperthyroid castrate male serum invariably produced vaginal canalization in the test mice and, with but two exceptions, ovarian and uterine response as well. Average ovarian response to serum injections of the first series would suggest that the hyperthyroid serum contained slightly more gonadotropic hormone than the normal, but this is due to high ovarian weights of

one litter, which through death is not represented in the control group. Omitting this, no difference between the two groups is indicated. Average ovarian weight of the assay mice in the second series is markedly greater than in the first, though the uninjected controls are slightly lighter. Seemingly, gonadotropic activity of the blood of castrate males may continue to increase for some time after gonadectomy.

TABLE 4

Response of immature female mice to serum of normal and hyperthyroid castrate male rats

LITTER NO.	NORMAL DONORS		HYPERTHYROID DONORS		UNINJECTED CONTROLS	
	Ovarian weight	Uterine weight	Ovarian weight	Uterine weight	Ovarian weight	Uterine weight
A. Injected immediately after castration						
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
1	5.5	69.7	..	...		
2	..	...	9.4	76.7	3.0	7.4
			6.7	58.6		
			9.3	68.5		
3	5.0	37.2	5.7	45.6		
			5.0	44.5		
4	4.2	44.5	3.2	43.8	3.4	8.9
			3.9	41.6	3.5	12.0
5	4.5	61.2	4.3	35.2	5.2	22.6
			3.6	10.1	3.6	28.0
6	4.1	38.1	4.5	22.8	3.1	13.1
			4.6	30.7	4.1	15.2
7	6.0	75.5	4.9	20.0	5.1	22.8
Average	4.9	54.4	5.4	41.5	3.9	16.2
B. Injections started 60 to 75 days after castration						
	6.4	45.1	5.4	39.0	3.0	7.0
	13.2	59.7	8.5	63.5	3.6	9.0
	7.8	57.3	4.5	19.4		
	7.5	37.5	6.7	38.0	3.8	16.3
			6.1	55.4		
Average	8.7	49.9	6.2	43.1	3.5	10.8

Vaginal canalization took place in all injected animals.

Ovarian response to the normal castrate male serum is greater than to the hyperthyroid in this series but this is largely due to one animal.

Apparently hyperthyroidism does not greatly affect the amount of gonadotropic hormone in the blood. Therefore such postulates as excessive excretion or destruction of this hormone do not account either for testicular hypofunction, or failure of the testis to respond to injection of gonadotropic hormones in hyperthyroid animals.

DISCUSSION

Experiments reported here clearly demonstrate that injections of thyroxin or feeding of thyroid gland has a deleterious effect on both the condition of the accessories and sperm production. Hypogonadism is apparent not only in those groups receiving such large amounts of thyroxin as to affect their general body size, but also in those receiving such minimal doses as to cause no weight loss. This is of especial significance in relation to the concept that any adverse effect that thyroid feeding has on the reproductive system is secondary to the deleterious effects on the body as a whole. In these experiments the effect on the reproductive system is roughly proportional to dosage and loss of body weight, but is independent of a general body decline.

The castrate condition of the accessories does not seem entirely explained by decreased effectiveness of testis hormone since 5 units of male hormone gave, in a few instances, slight stimulation in the hyperthyroid castrate. A reduction of this degree in the effectiveness of male hormone suggests that the amount secreted also is reduced. This explanation accounts for the marked depression of the accessories of hyperthyroid males.

Groups receiving the lighter thyroid dosages reveal an interesting difference in the reaction of gametogenesis and accessories to hyperthyroidism. Sperm production of groups receiving lighter thyroid dosage was but slightly affected, whereas their accessories were of castrate type. Since hyperthyroidism decreases effectiveness of both gonadotropic and

testis hormone, the accessories must reflect a decrease in amount as well as potency of testis hormone. Sperm production, however, is affected only by the gonadotropic action, thus explaining the greater sensitivity of the accessories to hyperthyroidism.

It would seem that testicular suppression in hyperthyroidism is due in part to reduction in available gonadotropic hormone. The reports of Evans and Simpson ('30), Van Horn ('31) and Cohen ('35) that thyroid feeding caused an increased gonadotropic potency of the hypophysis of male and female rats, removes the possibility that a deficient pituitary gland is responsible for the testis condition. Indeed, Van Horn suggested that the increase in anterior lobe potency of the female rat was in reality due to decrease in ovarian hormone, making the pituitary change a secondary one. A morphological basis for the increased potency of these hypophyses has been reported (Severinghaus, Smelser and Clark, '34). The basophiles were of maximum size, markedly increased in number, and castration cells numerous. The hyperthyroid rat basophiles resemble those of a castrate. The injection of gonadotropic extracts prevented the castration-like changes in the accessories and the structural alterations in the hypophysis. (Unpublished observation of Severinghaus and Smelser). Apparently basophilic pituitary changes in hyperthyroidism are secondary, due to decreased testicular activity.

The paradox of a hypofunctioning testis in the presence of an excessively potent pituitary gland may be explained by the decrease in the effect of gonadotropic hormones in hyperthyroid animals. Schockaert ('31) and Leonard and Hansen ('36) showed that certain gonadotropic hormones were more effective in thyroidectomized animals, and conversely, that they are less effective in hyperthyroid female rats, was demonstrated by Fluhmann ('34) and Tyndale and Levin ('37). The present study extends this finding to the male. Small dosages of gonadotropic hormones are without effect on the testis weight of hyperthyroid animals, although large amounts

(25 R.U. daily) of Antuitrin S maintained the testes of hyperthyroid animals. Since the condition of the accessories cannot be taken as reflecting only interstitial cell activity, because of the decreased effectiveness of testis hormone, decisions regarding action of pituitary hormones on the interstitial cells of hyperthyroid animals must rest on rather an insecure foundation. Examination of histological sections suggests that both the tubules and interstitial cells are involved in hyperthyroidism. The sensitivity of the accessories to small amounts of thyroxin and the action of male hormone in hyperthyroid castrates likewise support this view.

Reiss and Perény ('28), Van Horn ('31, '33) showed that thyroid feeding increased threefold the amount of estrin necessary to produce vaginal cornification in the spayed rat. A comparable, though more extreme, situation exists relative to the male sex hormone, for a daily injection of 5 B.U. had little or no effect on the hyperthyroid castrate, whereas, in the normal its effect is appreciable both grossly and histologically. Thyroxin is without effect on the accessories of castrates, indicating that atrophic accessories of hyperthyroid animals are produced through modification of their response to endocrines, and not through a change in their basic morphology.

The present data do not offer any explanations of the cause of decreased response to male hormone in hyperthyroidism.

The determination of the presence of gonadotropic material in the blood of hyperthyroid animals offers an explanation of the mechanism by which experimental hyperthyroidism affects the testis. The data show that, even in the extreme hyperthyroidism produced in these cases, amounts of gonadotropic material were found in the blood of hyperthyroid animals equal to that circulating in normal castrate litter mate controls. These facts dispose of the postulates that the gonad condition is due to such hormones being excreted or destroyed at an excessive rate. With the elimination of these hypotheses, the most reasonable conclusion seems to be that thyroid administration decreases the reactivity of the end organ, the

testis, or raised the testicular threshold of response to gonadotropic hormone.

The contradictory reports concerning the effects of hyperthyroidism can, in a large measure, be harmonized on the basis of these experiments. The effect of thyroxin on testis weight ranged from a slight gain to a marked loss, depending upon the dosage used, and suggest that some of the conflicting reports are due to differences in degree of the hyperthyroidism induced. Maintenance of the gametogenesis and accessories at nearly normal levels in hyperthyroid animals by the injection of gonadotropic material fits in well with the report of Abelin and Wiedmer ('32) that administration of gonadotropic extract produced vaginal cornification during the diestrus induced by hyperthyroidism.

It is extremely difficult to correlate some of the conditions existing in the hyperthyroid male and female rat. In the male the gonad picture is one of decreased secretion of glands and effectiveness of hormones. Thyroid feeding lengthens the estrous cycle of the female (Cameron and Amies, '26; Wang, '27; and Van Horn, '31, '33), but this is due to an increase in the function of the corpus luteum (Weichert, '30). Weichert and Boyd ('33) suggested that an increase in pituitary secretion might be one explanation of the active corpora lutea. No such increase is noted in the gonadotropic content of the blood of hyperthyroid castrate males.

In conclusion, it may be said that experimentally induced hyperthyroidism in male rats adversely affects the reproductive system. Hyperthyroidism acts in at least two ways. First, the testis hormone present is less effective, causing reduced secretory activity of accessory organs and castration changes in the hypophysis. Second, the gonadotropic hormones are less effective resulting in a reduction in number of sperm and amount of testis hormone produced. The latter is brought about by decreased reactivity of the end organ.

SUMMARY

1. Administration of thyroxin or dried thyroid gland causes a decrease in testis weight, sperm production, and hormone secretion, which is not due to injury to the body as a whole.

2. If thyroxin treatment is discontinued a rapid and complete recovery ensues.

3. Normal testicular function may be maintained during the hyperthyroid period by injection of gonadotropic extracts.

4. There is marked decrease in effectiveness of gonadotropic hormones in hyperthyroid males. Both hormones seem similarly affected, though evidence is more conclusive relative to the gametogenetic factor.

5. The amount of testis hormone required to stimulate the accessories of the castrate rat is increased in hyperthyroid animals.

6. Hyperthyroidism does not affect the amount of gonadotropic hormone found in the blood of castrates, thus indicating a normal secretion rate, and that excretion or destruction of the hormones is not exaggerated.

7. Testicular hypofunction in hyperthyroidism may be explained by a decrease in the ability of the testis to respond to pituitary hormones, or an increase in its threshold to gonadotropic hormones.

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A TESTIS STIMULATING EXTRACT OF BREWER'S YEAST ¹

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ONE PLATE (SEVEN FIGURES)

Friedman and Friedman ('34) reported the extraction of a substance from alfalfa meal which would induce ovulation in oestrous rabbits, and Peysahkovitch ('33) obtained an extract from Russian onions which stimulated the development of graafian follicles and corpora lutea in the ovaries of immature rats and mice. Fevold, Hisaw and Grep ('36) found that an extract of yeast would induce ovulation in oestrous rabbits and that when combined with pituitary FSH and injected into immature female rats the ovaries attained a larger size than would have been expected from the FSH acting alone. These reactions, however, were duplicated with copper salts and it was found that the yeast contained some copper.

The present report deals with a substance extracted from yeast which has a marked stimulatory action on the male gonads. Live Brewer's yeast was treated with several volumes of acetone and then dried to a powder. The extraction of this powder was carried out by the same method as described by Fevold et al. ('33) for the preparation of an unfractionated extract of dried pituitary glands. This procedure yielded an amber colored solution which could be administered subcutaneously, intraperitoneally or intravenously without ill effects. The assay has been made on immature male pigeons (4 to 5 weeks old), normal 21-day-old male rats and hypophysectomized immature and adult male rats.

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RESULTS

The immature male pigeon was indicated as a test animal due to the great sensitivity of their gonads toward pituitary gonad stimulating substance (Riddle, '31; Evans and Simpson, '34). Adequately large doses of yeast given over 7 days were found to produce testes distinctly heavier than those of control animals of the same age (table 1). This reaction could not be duplicated with copper acetate or boiled yeast extract. Microscopic study of the pigeon gonads showed that the hypertrophy induced by the yeast was due primarily to development of the seminiferous tubules. In control

TABLE 1
Immature male pigeons treated with yeast or copper acetate

TREATMENT	DOSAGE	DAYS	NUMBER OF BIRDS	AVERAGE WEIGHT OF TESTES	RANGE
	<i>gm. equiv.</i>			<i>mg.</i>	
Controls			12	24	16- 31
Yeast	6	7	5	73	46-115
	10	7	8	69	54- 84
Yeast (boiled)	6	7	5	24	13- 38
	10	7	5	24	19- 33
Copper acetate	(10 mg.)	7	4	26	22- 28

animals the tubules were poorly developed and in some instances had not yet acquired a distinct lumen.

Immature male rats 28 days of age were hypophysectomized and started immediately on yeast therapy (0.25 gm. equivalent of yeast powder daily). After 7 days of treatment the testicles of these animals were enlarged and scrotal, whereas operated controls showed the expected testicular regression. Histological examination gave evidence of essentially normal development of the tubules in the treated animals (fig. 2). The walls of the tubules were several cell layers thick in contrast with the pycnotic nuclei and thin walled tubules in the hypophysectomized untreated animals (fig. 1). The interstitial cells, however, appeared inactive and the atrophic secondary sexual structures (table 2) indicated a lack of stimulation.

Boiling the yeast extract completely inactivated its testicular stimulating property.

These observations were extended to the hypophysectomized sexually adult male rats to ascertain what influence the yeast might have on the gametogenic activity of the male gonad. The treatment (1 gm. equivalent yeast powder daily)

TABLE 2

Effect of yeast extract on the testes of hypophysectomized immature male rats

	HYPOPHYSECTOMIZED 6 DAYS	YEAST EXTRACT	YEAST EXTRACT BOILED 4 HOURS
Testes	195 ¹	395	193
Seminal vesicles	7.8	7	8.4

¹ Each figure represents an average of four to six animals.

TABLE 3

Effect of yeast extract on hypophysectomized adult male rats

	TESTES	SEMINAL VESICLES	PROSTATE
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Hypophysectomized	705	88	28
16 days (controls)	740	70	
	557	85	29
Hypophysectomized	2450	450	175
plus yeast 16 days	2613	720	215
	2230	280	150
	3080	400	290
Normal males untreated	2630	1260	460
	2710	860	340
	2805	770	250
	2770	1020	355

was started at the time of hypophysectomy. The results here were most striking. The testes of the treated animals remained firm and scrotal and at autopsy on the sixteenth day the testicles weighed well within the range of those normal untreated males of comparable age (table 3). The seminal vesicles and prostate glands, however, were not fully maintained as they weighed only about one-half as much as did

those of the normal males, yet they were many times larger than the degenerate accessories of the hypophysectomized controls which indicates that a fair amount of interstitial cell activity had been maintained by the yeast extract.

The seminiferous tubules of these treated males presented a remarkably normal picture histologically (figs. 3, 4 and 5). The investiture of the tubules could not be distinguished from that of normal males. That the yeast had not only sustained the tubules but was actively promoting the maturation of gametes was attested by the presence of dividing cells among the various recognized stages of sperm cell formation. The epididymides of the treated animals were filled with sperm (figs. 6 and 7).

These animals did not copulate nor exhibit any interest in receptive females; a continence probably due not to a lack of potentialities for reproduction but to the severe physical debilities resulting from hypophyseal deprivation. The symptoms of cachexia hypophyseo-privia were not alleviated in any way by yeast therapy. The weight of the thyroid and adrenals in the treated animals were slightly though not significantly greater than those of untreated operated controls.

Four adult males were treated with yeast for 10 days beginning on the tenth day after hypophysectomy. Their gonads and accessory sexual structures were compared with those of operated controls killed 10 and 20 days post operative. The yeast did not forestall the progressive testicular regression although it did slow these processes slightly (table 4).

Normal immature male rats also respond readily to yeast stimulation. Twenty-one day old male rats of selected uniform body weight were treated with graded dosages for periods of 6 or 12 days (table 5). It was of considerable interest to find that the gonadal response was dependent upon the dosage given within physiological limits, thus simulating the response of the male to hypophyseal preparations. The weight of the seminal vesicles taken as a criterion of interstitial cell secretion shows that small and moderate dosages

of yeast did not induce hyperactivity of these cells but the extremely high dose did produce a weak stimulation as evidenced by the increased size of the accessories. Microscopic evidence of interstitial cell hypertrophy, however, was lacking. The tubules on the other hand responded to the yeast by gross enlargement which was due entirely to proliferation without maturation of the germinal cells.

TABLE 4

Effect of yeast extract on young adult male rats 10 days after hypophysectomy (1 gm. equivalent yeast powder daily for 10 days)

	TESTES	SEMINAL VESICLES	VENTRAL PROSTATE
	mg.	mg.	mg.
Normal males untreated	2213 ¹	164	93
Hypophysectomized 10 days (untreated)	1465	34	23
Hypophysectomized 20 days, yeast last 10 days	675	28	29
Hypophysectomized 20 days (untreated)	483	37	15

¹ Each figure represents an average of three or four animals.

TABLE 5

Effect of yeast extract on normal 21-day-old male rats

NUMBER OF ANIMALS	AGE AT AUTOPSY	DOSAGE PER DAY	DURATION DAYS	TESTES	SEMINAL VESICLES
	days	gm. equiv.		mg.	mg.
3	27	Controls		199	6.0
3	27	0.25 ¹	6	378	6.6
3	33	Controls		357	8.2
3	33	0.08	12	530	8.3
3	33	0.33	12	643	8.5
3	33	1.00	12	810	21.0

¹ Dosages are given in terms of grams equivalent of dried yeast powder.

Females

The ovaries of immature rats did not respond to the yeast extract; the genital tracts remained infantile and the vaginae did not open. It is known that the gonad stimulating action of pregnancy prolan can be greatly augmented by the addition of a trace of follicle stimulating hormone (Leonard, '32; Fevold and Hisaw, '34). A parallel substitution of yeast extract for FSH, however, did not result in any enhancement of the ovarian effect.

SUMMARY

An extract was prepared from desiccated yeast which possessed the property of stimulating testicular growth in immature rats and pigeons and supported gametogenesis for at least 16 days following pituitary ablation in adult male rats. In the rats the testicles remained scrotal; the accessory sexual organs were poorly stimulated in the immature animals and only partially sustained in the hypophysectomized adults.

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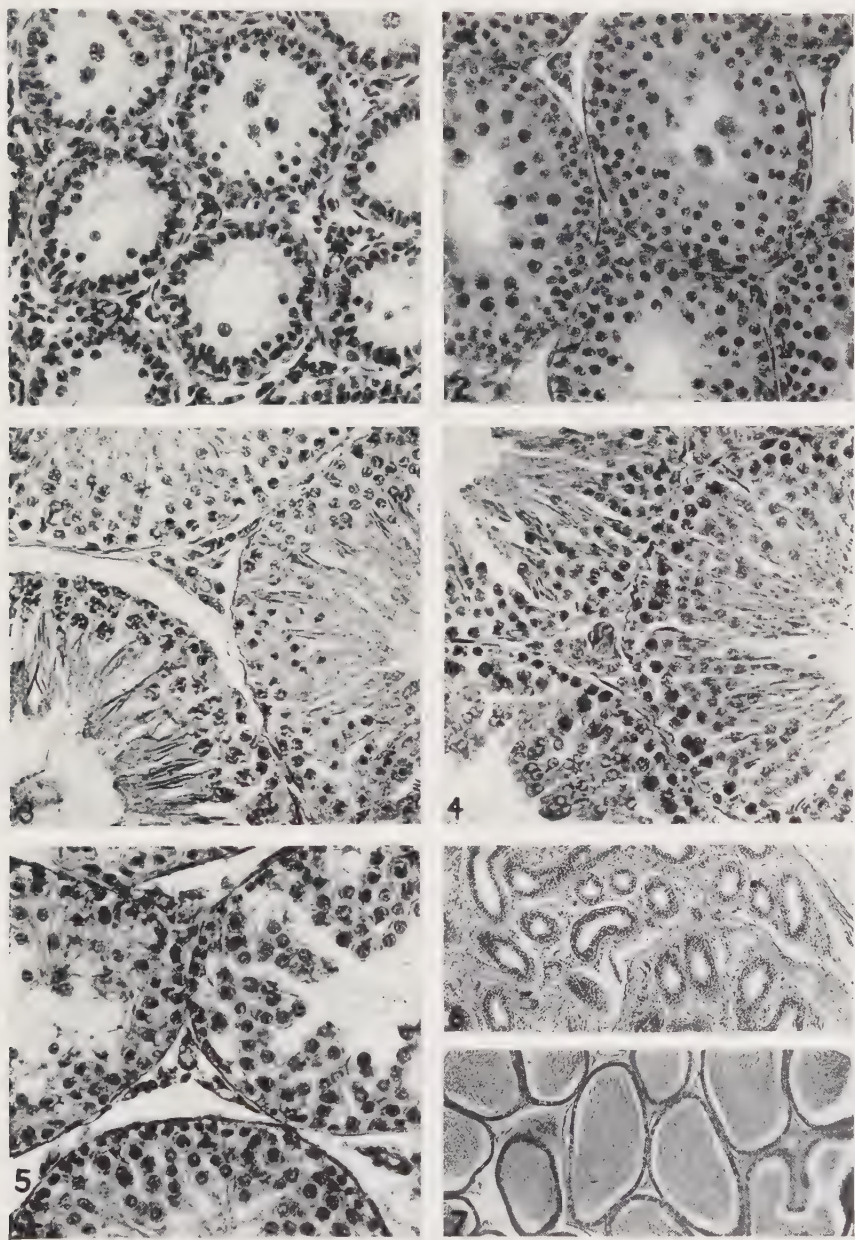
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PLATE

PLATE 1

EXPLANATION OF FIGURES

- 1 Testis of a rat hypophysectomized at 28 days of age and autopsied 7 days later. $\times 175$.
- 2 Testis of a rat hypophysectomized at 28 days of age and treated for the following 7 days with yeast extract. $\times 175$.
- 3 Testis of a normal adult rat. $\times 330$.
- 4 Testis of an adult rat hypophysectomized and treated for the following 16 days with yeast extract. $\times 330$.
- 5 Testis taken from an adult rat 16 days after pituitary ablation. $\times 330$.
- 6 Section of an epididymis taken from an adult rat 16 days after pituitary ablation.
- 7 Epididymis of an adult rat hypophysectomized and treated for the following 16 days with yeast extract.



OBSERVATIONS ON THE FORMATION OF NEW FOLLICLES IN LIVING GRAFTS OF THE THYROID GLAND IN RABBITS ¹

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TWO FIGURES

The formation of new follicles in the thyroid gland has been extensively investigated and like many other things concerning the gland there is lack of agreement about the process. In foetal life after the primary follicles have formed, secondary follicles arise from them by solid buds, by hollow buds and by constrictions, Norris ('16, '18). As for the mature animal, Wilson ('27) believes that follicles are propagated by budding but not by constriction. Cowdry ('38) states definitely that new follicles are formed by budding and that in adult life this is the chief manner in which they are formed. Marine ('32) believes that within the thyroid there are follicular rests and small follicles which may be considered as new follicles or buds in various stages of development. But there are those who maintain that budding plays no part in the thyroid and who doubt that new follicles are formed at all in the mature gland. Uhlenhuth and Karns ('27) found that in *Amblystoma maculatum* growth of the whole thyroid takes place not by division of large follicles into smaller ones or by budding but by increased size of the primary follicle. The development of follicles from undifferentiated cells between follicles, or from cells which have wandered away from formed follicles, or from cells left after

¹ This work was supported in part by a grant from the Penrose Fund of the American Philosophical Society.

the disintegration of follicles (Zechel, '33), has also been described or the possibility suggested. The existence of undifferentiated cells between the follicles has been denied by Rienhoff ('31). Severinghaus ('33) described intrafollicular follicle formation after activation of the thyroid by the thyreotropic factor of the pituitary gland.

The method used in this study was the transparent chamber technique of Clark which had been adapted for the study of transplanted tissue by incorporating a device in the chamber whereby the living contents could be directly exposed, under fluid, the grafts placed thereon and the cover reapplied without appreciable injury to the blood vessels (Williams, '34, '37). The grafts were in all case autogenous. They varied in size from $50\ \mu$ to $300\ \mu$ in diameter. The preparation and implantation of the grafts was done under a dissecting microscope. When grafts of this size are placed in contact with blood vessels and no more than $\frac{1}{2}$ hour elapses from the time of their removal from the neck till they are placed in the new environment, they will, in my experience, invariably survive. The same individual follicles were examined daily with a $100\times$ special oil immersion lens and a $5\times$ ocular or with various lens combinations of lower magnification. Thirty grafts in six rabbits were studied. Five of the animals were males and one a female. They were all mature and varied in age from 2 to $2\frac{1}{2}$ years.

Approximately 2000 individual follicles in the six rabbits were observed. Of this number 200 were followed in detail by camera lucida tracings and photomicrographs at various intervals for periods varying from 3 months to 1 year.

The formation of new follicles was of rare occurrence and when it did occur it was always after injury. In one case all the thyroid was removed except the grafts in the ear. This animal had been studied daily before the operation for many weeks and for 3 weeks thereafter and although the follicles changed, much as the thyroid generally responds to injections of the thyreotropic factor of the pituitary gland, no new follicles formed. In another case about three-quarters of the

thyroid was removed. The grafts in this animal had been observed for 3 months before the operation and were studied for 7 months after it, but in this case too no new follicles formed.

The first examples of new follicle formation were obtained in the area of which figure 2 E is a composite photomicrograph. This area was originally the lateral margin of a graft and was, therefore, the region where the actual cutting involved in preparing the graft had been done. All the follicles were destroyed as such but some time after the disturbances associated with the grafting operation had subsided a large mass of closely packed cells could be observed which had once formed the follicles which occupied this region. When first noticed they were surrounded by a definite connective tissue capsule. This capsule is visible in figure 2 E and appears there much as it did before the follicles began to form. The cells composing this mass were so refractile that the capsule surrounding them was much more conspicuous than they were. Because of this the nature of the mass was not recognized immediately, it having more the appearance of a lymphatic vessel which had been cut off than anything else. Then within this enclosed region small areas appeared (fig. 1, 4/25) the refractility of which was much greater than elsewhere and upon close inspection with the oil immersion lens the boundaries of the cells limiting the clear areas could be seen. The junctions of cells were not visible with the method as originally used, but with certain improvements in the technique they sometimes become so. A description of these improvements is being prepared for separate publication, together with the details of studies concerning the cell boundaries and other things.

The day following the appearance just described most of the highly refractile areas, which were considered to be colloid, had disappeared and those that were present were in locations different from the preexisting ones (fig. 1, 4/26). Between 4/26 and 5/9, figure 1, various islands of colloid appeared and disappeared but always at random, never appearing in exactly the same position twice in succession. At

5/9 three masses of colloid appeared in a row (fig. 1). The cells composing the walls were clearly defined and the entire arrangement appeared about as ordinary follicles do, except that no connective tissue separated them from adjacent cells. The cells intervening between the colloid masses began to flatten out and disappear. As shown, at 5/13, figure 1, the masses fused to form a single large one. It was then observed that a connective tissue extension from the outside capsule was invading the cells and surrounding those lining

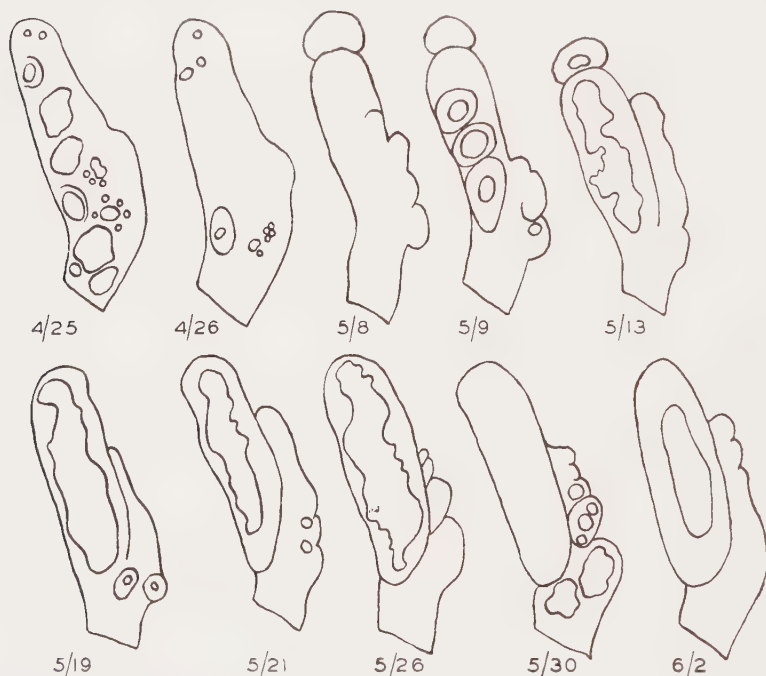


Fig. 1 Selected camera lucida tracings of the area in figure 2 included within the bracket. The outside line represents the connective tissue capsule enclosing a large group of thyroid cells. Individual cells are not shown. The enclosed areas within the capsule represent masses of colloid. The successive tracings illustrate the appearance and disappearance of the colloid at random among the cells, the fusion of three colloid masses, 5/9, to form a single mass, 5/13 and the segregation of this mass and its surrounding cells by the growth of connective tissue from the periphery. At 5/8 and 5/30 all visible colloid had been discharged. The figures below the tracings refer to days of the month. $\times 269$ (original magnification $\times 500$).

the large collection of colloid. By 5/21, figure 1, this had grown completely around, isolating the included cells from other cells and thereby forming a permanent follicle. The colloid in this follicle increased and decreased, even disappearing completely at times (fig. 1, 5/30) but 5 months later it was still a follicle and never again has it presented an appearance like that shown at 4/25, figure 1. The other follicles in figure 2 E followed the same course as that of the one described. In no case was a permanent follicle formed unless the connective tissue from the periphery had grown in and formed a complete capsule around it individually.

Attempts were made to stain follicles with methylene blue by making a hole in the mica cover slip, flooding the chamber with an 0.02% solution of the stain and allowing it to diffuse through the hole. As a staining procedure this was practically a failure. However, in one specimen the hole in the cover was made so close to a graft that the trauma plus the toxic action of the dye caused the follicles in the neighborhood of the hole to disintegrate. The first reaction, which came on about 12 hours after the application of the stain, was a swelling of the connective tissue around the follicles and swelling of the walls. This was followed by a complete disappearance of colloid and a loss of all recognizable follicular structure. The edema and leucocytic infiltration caused an almost complete loss of visibility in the affected region. When this subdivided, 4 days later, the former location of the original follicles could be determined from the distribution of vessels, inert foreign matter (which always helps orientation) and from the adjacent unaffected follicles. No semblance of a follicle remained in the affected region, but among the blood vessels were islands of cells the boundaries of which were distinct and left no doubt that they were thyroid cells. At first there was no connective tissue reaction but this quickly set in and a ring was formed around each group of cells. From this point on the sequence of events was a repetition of that recounted previously. Colloid appeared and disappeared among the cells. Connective tissue trabeculae invaded

the cell mass and whenever they formed a capsule around a group of cells arranged in such a manner that they were only one cell thick around the colloid then the morphology of that follicle did not change appreciably later and a permanent follicle had formed.

In some follicles which were further removed from the source of injury disintegration did not take place. They did, however, show swelling of the cells but without much loss of colloid. At one or two places on some of these follicles the cells divided within the follicular capsule and at these places the wall was two or more cells thick. Colloid formed between these cells and it appeared that a clear cut case of intra-follicular follicle formation had been observed (fig. 2 A). In every case of this kind, and there were many of them, this was a temporary condition. The cells or portion of cells which had visible colloid on two surfaces—on one side the colloid of the original follicle and on the other the colloid of the apparently new formed follicle—in every case disappeared and the first follicle returned to its original condition.

Figure 2 B is another apparently clear cut case of intra-follicular follicle formation. This one was produced by injecting large quantities of the thyrotropic factor of the

Fig. 2 A, photomicrograph of a portion of the wall of a large follicle which had been injured with methylene blue. This injury caused the cells at the point illustrated to divide and what appears to be a new follicle to form. The wall between the two masses of colloid disappeared and the original follicle returned to its previous condition. B, a photomicrograph illustrating three masses of colloid within one follicle resulting from the injection into the animal of large quantities of the thyrotropic factor of the pituitary gland. C, a photomicrograph of the follicle at B 3 days later, after the stimulation had subsided, illustrating that in these cases intrafollicular formation of new follicles is a pseudo-formation. D, a photomicrograph of a portion of the wall of a follicle having foldings in it from which buds might form. These have never been seen to occur. At the left are tracings of the follicles shown in the photographs. E, a composite photomicrograph of a large group of thyroid cells left after the disintegration of follicles following injury. Individual cells cannot be seen in the photograph. A connective tissue capsule surrounds the group. Within it colloid masses appear. These come and go as shown in figure 1, until connective tissue grows in and segregates the cells into smaller groups. The portion within the bracket is illustrated on successive days in figure 1. This photograph fits into figure 1 at 5/25. $\times 375$ (original magnification $\times 500$).

pituitary gland into the animal over a considerable period of time. The cells of this follicle hypertrophied and the colloid completely disappeared in the way that many follicles respond to this hormone. When colloid appeared again it was in three separate places inside the original capsule. This, too, is a temporary condition and rarely lasts for more than 2 days.

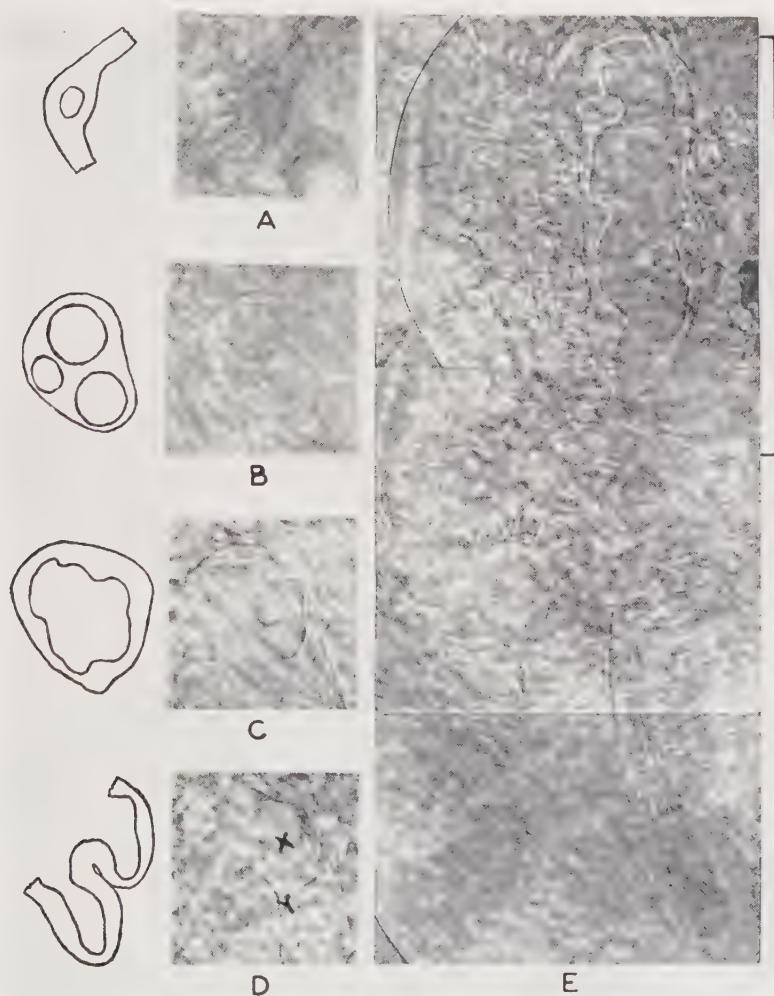


Figure 2

This, and all similar cases observed, subsided to a condition unrecognizable from that of the original follicle. Figure 2 C is a photograph of the follicle after it had returned to its original condition.

In all grafts there are a few follicles like that illustrated in figure 2 D. The foldings of the wall are not such as are seen after prolonged administration of the thyreotropic factor of the pituitary gland. The follicle illustrated, and others like it, was followed daily for months with the expectation that a portion of it would be pinched off at either X or Y and form a new follicle by budding. Such follicles frequently come so close to pinching off a portion of themselves that one feels certain it has taken place, but if followed long enough it becomes apparent that no reproduction had occurred. Only following disintegration of follicles as the result of some mechanical or chemical injury have new follicles formed and then they did so from islands of cells in the same place where the original follicle had been. No undifferentiated cells, which are said to be normal constituents of the gland, have been recognized between the follicles of a graft.

DISCUSSION

In this study, permanent new follicles formed in only one way; namely, from islands of cells lying among the blood vessels and follicles. Some of the cells were originally a part of follicles which had disintegrated as the result of injury. The interfollicular cells described in the literature as normal constituents of the gland may or may not have also played a part; there is no evidence here on that point. Rienhoff ('31) does not believe such cells exist but this seems an extreme view. No new permanent follicles were formed unless the cells composing them had first been surrounded by a capsule. It is presumed that this capsule is of connective tissue origin. One may define a follicle then as a single layer of thyroid cells surrounded by a capsule and enclosing colloid. This capsule cannot properly be considered as only a part of the stroma of the gland. It is essential to the integrity of the

follicle and when it disappears as the result of injury the follicle, colloid and all, disappears as a recognizable unit and the cells exist as inactive islands among the blood vessels. The capsule is clearly recognizable in living grafts and is more prominent than connective tissue elsewhere, although it may appear in sections as simply reticular connective tissue. Budding from parent follicles, whether solid or hollow (Norris, '16; Wilson, '27) was not observed. Such processes may take place in the foetus or in the young rabbit, but if they occur at all in mature rabbits, they must be rare—that is, if the study of the grafted gland is an adequate criterion. Manley and Marine ('15) and others found that autogenous grafts of thyroid tissue were not recognizably different either physiologically or histologically from the normal gland.

Pseudo-intrafollicular follicle formation in grafts occurred only after stimulation, whether by injury or by administration of the thyreotropic factor of the pituitary gland. It was always a temporary condition and lasted about 2 days. It is conceivable that if it lasted longer the connective tissue could grow in and form a permanent follicle but no case was observed in which it did so. Severinghaus ('33), using fixing material, described such cases.

Zechel ('31) states that, "The follicle is not a permanent structure but merely a phase in structural metamorphosis which alternates between two extremes, the follicle on one end and the interfollicular cell group on the other." If this concept is true for the rabbit, then the period of alternation must be greater than 1 year, because follicles have been studied for such a period and no disintegration has been observed except in the presence of injury. In the absence of local injury the production of new follicles in thyroid grafts did not occur even after situations presumably favorable for their production had been created, such as following the removal of large portions of the thyroid gland.

Marine ('32) states, "In the dog, if one removes $\frac{3}{4}$ of the gland ordinarily regeneration occurs in the remaining portion; but if small amounts of iodine are given such regeneration does not take place. If more than $\frac{3}{4}$ of the gland is

removed iodine will not protect against regeneration, but desiccated thyroid will still protect.” In this study extensive removal of the thyroid was done in only two cases. The grafts in one of these was followed for 7 months and the other for 3 weeks but no regeneration was observed. All animals were kept on the same diet, which was adequate in iodine. This, together with the fact that in both cases some thyroid tissue remained in the animals which may have been within the three-quarter limits prescribed by Marine, may account for the failure of regeneration to occur in these grafts as he described in the dog.

Before permanent follicles are produced from islands of thyroid cells some of the cells are capable of producing colloid which appears and disappears at random among them. The cells producing the colloid may be separated from the nearest blood vessels by several thyroid cells not concerned in the colloid production. In such cases when the colloid disappeared it must have either been incorporated in the lining cells or escaped between them. Before it could get to the vessels, if it was destined for them at all, it must have passed between cells or have undergone a process of absorption by several cells until it reached some which were in contact with vessels. It seems more likely to me that it passed between the cells, for the adjacent cells did not show the increase in refractility which one would expect if they had just absorbed a large quantity of colloid. This speculation is pertinent to the question of colloid release and will be elaborated in another place.

The foregoing results establish one thing; namely, that new follicles can form in grafts of the thyroid glands from cells some of which at least were once a part of other follicles which had disintegrated from injury, and that they will be permanent follicles only if surrounded by a capsule which is probably of connective tissue origin. They suggest that intrafollicular follicle formation is a temporary condition resulting from excessive stimulation of the gland and that budding from preexisting follicles, if it occurs at all in the mature normal animal, is a rare process.

SUMMARY

New follicles formed in living grafts of the thyroid gland of rabbits from groups of cells left after the disintegration of follicles. The disintegration was always the result of injury. Such thyroid cells stimulated the formation of a connective tissue capsule around them. Among the cells colloid appeared and disappeared at random. Connective tissue grew between the cells and separated them into smaller groups. When the connective tissue separation occurred in such a manner that the cells were only one layer thick around the mass of colloid, then a new follicle was formed and as long as the capsule was intact it remained a follicle and colloid never appeared in it, except in its center. In the specimens studied intrafollicular follicle formation was a temporary condition, seen only in stimulated glands. Reproduction by budding was not observed.

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RESULTS OF ATTEMPTED TOTAL MAMMECTOMY IN MICE OF A HIGH TUMOR STRAIN ¹

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THREE FIGURES

In order to ascertain whether the absence of the mammary glands would have any influence on the estrous cycle, Long and Evans ('22) removed the glands of rats within the first 8 days after birth. They state that at this time the gland is confined to an area within 1 or 2 mm. of the nipple, and its extirpation can be easily carried out under the binocular microscope. The animals mature as do normal ones, and their estrous cycle is normal. Five individuals in which the mammary apparatus was completely removed were allowed to mate; the resulting pregnancy was normal in every respect.

A similar experiment was undertaken in the highly inbred dilute brown strain of mice, in which the incidence of mammary gland tumor in the breeding females is very high. It was thought that it would be possible to determine whether abnormal growth in other parts of the body, very rarely observed in animals of this strain, would replace those ordinarily located in the mammary glands, if and when these structures were removed.

To study the extent of the development of the mammary gland several animals between the ages of 5 and 10 days were killed and carefully skinned with the subcutaneous fat pads retained in normal position. After fixation the extent of the ducts was well visible with the aid of a dissecting microscope.

¹ This investigation has been aided by a grant from the National Research Council.

It was found that although the primary ducts projecting down from the nipples have a consistent direction of growth in the subcutaneous fat pads, the same cannot be said of the secondary branches. The length and the direction of growth of these is very irregular. The gland structure is not distinguishable from the surrounding adipose tissue in the living animal, and the extirpation would have to depend on the chance that removing small, round areas of the skin around each nipple, loosening the skin, and removing as much of the subcutaneous fat pads as could be reached with forceps would include the whole gland.

To ascertain what anesthesia would give best results and the youngest age at which animals would survive the mamnectomy, several trial operations were performed on mice

TABLE 1
Fate of 126 mice living beyond 30 days

		109 MICE LIVING OVER 6 MONTHS		
KILLED AT 6 TO 8 WEEKS	DIED BEFORE 6 MONTHS	Developed mammary gland tumors	Developed tumors of other origin	Died tumor free
5	12	81	9	19
		74.31%	8.25%	17.43%

between the ages of 5 to 10 days. Chilling in the refrigerator was tried but had to be abandoned. The removal of all ten glands took considerable time, and chilling did not render the animals motionless for sufficiently long periods, while repeated chilling killed them. Anesthesia with ether proved more successful. The youngest animals which were able to survive the operations were 10 days old. The mortality resulting from the operation even at this age, and from the fact that the mother refused to nurse the operated animals, was very high.

With much difficulty 126 operated mice were raised beyond weaning age (30 days). Table 1 summarizes the eventual fate of these animals.

Five mice were killed when 6 to 8 weeks old to ascertain whether the removal of the glands was successful. In these

animals the skin was carefully removed, fixed and examined under the dissecting microscope. In none of them was any gland structure visible.

However, it became evident from later observations that all the glands were not removed from all the animals, and a few cells left in accidentally, although not visible at 6 to 8 weeks, were sufficient to produce small isolated groups of lobules during subsequent pregnancies.

The operated animals were mated, the pregnant mice were separated into individual pens, and their litters recorded. As the mice were unable to nurse their offspring, they were put back in the breeding pens and soon became pregnant again. They had on the average one litter a month while unoperated females which were separated from the male during their nursing period had on the average one litter every 2 months.

When the animals were 6 months old one of them developed a tumor at the region of the fifth (caudal) mammary gland. The tumor was sectioned and on microscopic examination proved to be an adenocarcinoma of the mammary gland. Later many more tumors developed showing that the complete removal of the glands was not accomplished, although some glands were entirely, and all partially, removed in every animal.

As the youngest mouse which developed mammary gland tumor was 6 months old, this age was considered as threshold age, and twelve animals which died younger without developing tumors were disregarded.

Out of the remaining 109 animals 81 (74.31%) developed tumors of the mammary glands. All the tumors were sectioned and examined microscopically.

The removal of the right and left fifth glands (caudal pair) was the most difficult. The ducts of these glands are very close to the opening of the vagina and rectum, and the skin is not loose enough to allow the subcutaneous fat pads to be removed at a sufficiently large area.

Some of the animals had multiple tumors, and altogether ninety-five mammary gland tumors were observed. Fifty-three (55.78%) tumors out of these were on the fifth pair of glands, while forty-two (44.21%) were found distributed on the remaining four pair of glands.

To be able to compare these figures with the frequency of the position of tumor appearance of unoperated dilute brown animals, eighty-five mice were chosen at random and tabulated according to tumor position. Multiple tumors were numerous, and 140 mammary gland tumors were observed on the eighty-five mice. Only thirty-three tumors (23.57%) were

TABLE 2
Nine mice having tumors of non-mammary gland origin

LEDGER NO. OF MOUSE	AGE AT DEATH	DIAGNOSIS OF TUMORS
	<i>months</i>	
94	26	Carcinoma of lung
95	26	Fibrosarcoma of uterus
96	26	Subcutaneous hemangioma (mammary gland region)
196	19	Lymphoblastoma (generalized)
244	25	Papilloma of intestinal epithelium
1002	13	Osteogenic sarcoma of left femur
1090	16	Hemangioma of spleen
1091	22	Reticulo-endothelioma of a mesenteric lymph node and liver
1147	22	Fibrosarcoma of uterus and bilateral ovarian hemangioma

situated on the fifth pair of glands as compared with 55.78% in operated mice, and 107 (76.42%) were found on the remaining four pairs of glands. Evidently in the absence of the other glands the hereditary susceptibility to tumor formation was expressed in the glands which were present, showing an unusually large number of tumors on the fifth pair of glands.

Twenty-seven mice living longer than 6 months died eventually without developing mammary gland tumors. Nine of these animals, 8.25% of the total number, had tumors of other organs. Table 2 shows the age at death and the diagnosis of these tumors.

Nineteen animals (17.43%) died without developing tumors of any kind. Three of these animals died during parturition.

Figures 1, 2 and 3 show the number of individuals and age at death of those mice which developed mammary gland tumors, those which had tumors of non-mammary gland origin, and those which died tumor-free. The average age at death for mice with mammary tumors was 12.81 months; for mice with tumors of non-mammary gland origin 21.66 months; for mice which were tumor-free 17.21 months.



Fig. 1 Age at death of mice which developed mammary gland tumors.

The frequency of non-mammary gland tumors in the unoperated dilute brown female mice is much less than 1%. It is possible that the reason for this is the fact that the animals die with mammary gland tumors at a younger age.

Although the complete removal of the mammary gland was not accomplished in all cases, the amount of glandular tissue and consequently the number of cancer susceptible cells was greatly reduced. In spite of this 74.31% of the mice developed mammary gland tumors. It seems that the development of tumors was not noticeably influenced by the numerical decrease of cancer susceptible cells.

Two factors might have had favorable influence on the development of tumors:

1. Due to the fact that the operated animals were unable to nurse their offspring and were mated soon after the young were born, the number of pregnancies was increased. During pregnancy the glands attain a periodic maximum development. This development is brought about during the first

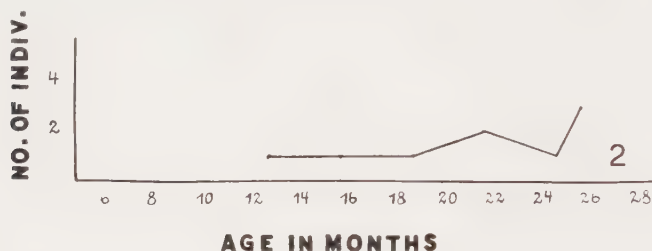


Fig. 2 Age at death of mice which developed tumors of non-mammary gland origin.

Fig. 3 Age at death of mice which died tumor-free.

part of pregnancy by ovarian hormones and results in an active cell division. During the second part of pregnancy an anterior pituitary hormone (prolactin) produces hypertrophy and functional activity of the glandular cells.

Previous comparative studies of the mammary glands of the dilute brown strain and the C57 black low tumor strain (Fekete, '38) seemed to indicate that the first signs of abnormalities occur at the time when active cell division ceases and secretion starts. The failure of small groups of epithelial cells in the mammary gland of some dilute brown mice to

respond to the stimulation of prolactin and persist in cell multiplication seemed to lead gradually to carcinomatous changes.

The greater number of pregnancies in the operated animals increased the chance of abnormal response to the hormonal stimulations.

2. Experimental work on the influence of blockage of the nipple by Bagg ('25), Bogen ('35), Fekete and Green ('36) seemed to indicate that the resulting milk stagnation had an influence on tumor development. The last two investigators cauterized all the nipples on the right side of dilute brown and C57 black animals. They found that tumors were more numerous and appeared at an earlier age on the blocked side in the genetically cancer susceptible dilute brown animals. Blockage itself did not cause tumors in the hereditarily resistant C57 black animals.

The operated animals had isolated masses of glandular tissue from which there was no outlet to the surface. Although none of the glands could be suckled, and all regressed after parturition, microscopic examination did show milk stagnation on some of the sections.

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THE MORPHOGENESIS OF THE HYPOPHYSIS IN CRYPTOBRANCHUS ALLEGHENIENSIS

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SIX TEXT FIGURES AND ONE PLATE (THREE FIGURES)

INTRODUCTION

Our knowledge of the amphibian hypophysis is derived mainly from the studies of Götte (1875), His (1892), Kingsley and Thyng ('04), Tilney ('11), Stendell ('13, '14), Atwell ('18, '21) and Sumi ('24, '26). By some of the earlier investigators, it was claimed that the epithelial portion of the hypophysis originated from the entodermal foregut; some thought that it received a contribution from the notochord. Only for the past 20 years has it been generally admitted that the early anlagen of the hypophysis are completely ectodermal, the epithelial hypophysis arising from the oral ectoderm and the neural portion evaginating from the brain. In Amphibia, the oral contribution is not a pocket as in mammals, but a solid plate of cells.

Few investigators have undertaken the study of the entire developmental history of the hypophysis in a single species of amphibian. As a result, several important questions have been left unsettled. Atwell ('21) states that his studies of the amphibian hypophysis were undertaken, in part, to answer the question as to the presence of a lobe homologous to the pars tuberalis of mammals, birds and reptiles, in the hypophysis of lower vertebrates. He investigated the development of the hypophysis in the Anura and in several tailed amphibians, but did not include *Cryptobranchus*, the largest of the native urodeles. No investigation of this subject has ever been carried out on *Cryptobranchus*.

¹From September 1938, in the Department of Anatomy, University of Minnesota College of Medicine.

I wish to express my gratitude to Prof. B. G. Smith, who obtained all the specimens of *Cryptobranchus* used in the investigation, and whose guidance made its completion possible. For further helpful suggestions, I also wish to thank Dr. A. T. Rasmussen and Dr. R. Blount, of The University of Minnesota.

MATERIALS AND METHODS

As compared with most amphibians, *Cryptobranchus* develops slowly. Hatching occurs about 6 weeks after the eggs are fertilized, the newly-hatched larvae measuring about 25 mm. in length. At the end of the first year, the smallest larvae taken from their natural environment measure about 75 mm. Metamorphosis occurs at the end of the second year, when the larvae have reached a length of at least 120 mm. Sexual maturity is attained with a length of about 300 mm., for the male, and 350 mm. for the female. The time required for development up to sexual maturity is not known, but is probably 4 to 5 years.

No single method of designating the stages of development is entirely satisfactory. The period prior to hatching has been divided by Smith ('12) into twenty-three stages, defined on a morphological basis. For these early stages, this method is more accurate than one based on age or length of body. For the larval period, a mere designation of the age in months appeared sufficient until it was found that for the first winter, larvae left in their natural environment develop more slowly than those reared under artificial conditions, but in the following spring and summer the reverse is true. After hatching, the length in millimeters affords a better index of the stage of development. The specimens selected for the present investigation range from embryonic stage 21 to metamorphosed individuals of about 400 mm. Some of the largest specimens, taken in early spring, would have attained maturity during the following autumnal breeding season. The remaining large specimens were completely matured.

The fixing fluids used, were Smith's bichromate-acetic-formalin, Bouin's, Tellyesnick's, Helly's, Zenker's and Lawdowsky's. The stains used throughout were haematoxylin and eosin. All specimens were embedded in paraffin. Transverse, frontal and sagittal sections were cut 10μ in thickness.

In several cases the entire brain was dissected out, drawn and photomicrographed. Camera lucida drawings of many sections were made. One series was used for graphic reconstruction of the hypophysis (fig. 6).

OBSERVATIONS

The hypophysis was examined grossly and microscopically in the stages mentioned below. Its lobes were studied in relation to each other and to the structures surrounding the hypophysis. In the embryonic stages one must depend almost entirely on the study of serial sections, but in the later stages the hypophysis is described as it appeared before sectioning and as it appeared upon microscopic examination.

In *Cryptobranchus* as in *Amblystoma* (Johnston, '10) no stomodaeum is formed. Instead, the ectoderm of the pharyngeal plate slowly thins out and disappears. This process is well under way in the stages represented by figures 1 and 2, but anteriorly, enough oral ectoderm remains to reveal its continuity with the epithelial hypophysis. In view of the careful work done by Johnston ('10) on earlier stages in *Amblystoma*, it was deemed unnecessary to study similar stages in *Cryptobranchus*. The entoderm of the pharyngeal plate persist until the time of hatching.

Stage 21. This stage is attained about 2 weeks before hatching. The specimens are 12 to 14 mm. in length, and the yolk mass is still large and greatly distended. The external gill rudiments are present as simple ridges. Small front limb buds are present, but there is no external indication of hind limb rudiments. Pigmentation is beginning, but is almost entirely confined to the dorsal surface. The pharyngeal plate is not yet perforated.

Upon sectioning, it becomes apparent that the epithelial portion of the hypophysis is present in an early phase of development (figs. 1 and 9). The stalk of the buccal anlage of the hypophysis is a thin cord of cells connecting the gland with the oral ectoderm. An ectodermal origin is clearly indicated by the character of the cells. The cells are darkly stained and appear to be the same as the cells of the ectoderm

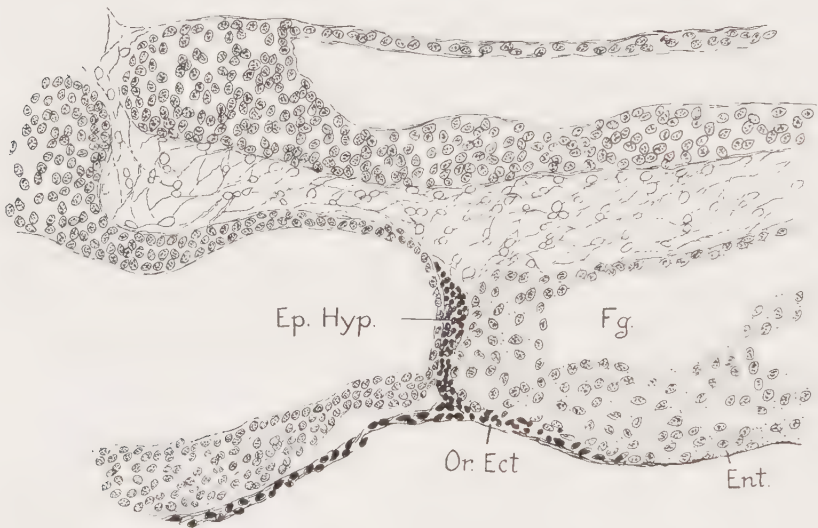


Fig. 1 Stage 21, *Cryptobranchus allegheniensis*. Camera lucida drawing of a sagittal section, showing the origin of the epithelial portion of the hypophysis from the oral ectoderm. The anterior end of the section is to the left. Ep.Hyp., epithelial hypophysis; Or.Ect., oral ectoderm; Ent., entoderm; Fg., foregut. $\times 100$.

of the oral region. The dorsal part or main body of the epithelial hypophysis is a broad plate of cells in close apposition with the pharyngeal entoderm. No differentiation of the neural portion of the hypophysis can be seen, though the epithelial portion and the brain wall (which will later form the neural portion) have come into apposition. The notochord does not extend far enough forward (cephalad) to come into relation with the hypophysis.

Stage 22. This is 1 week prior to hatching. The specimens are 15 to 22 mm. long. At this time the rudiments of the external gills are no longer simple ridges, but are branched. The front limb rudiments are conspicuous structures. Toward the end of this stage, small hind limb rudiments appear. The pharyngeal plate is not yet perforated.

The epithelial anlage of the hypophysis, which, as we have seen, is present in stage 21 as a plate of cells connected to

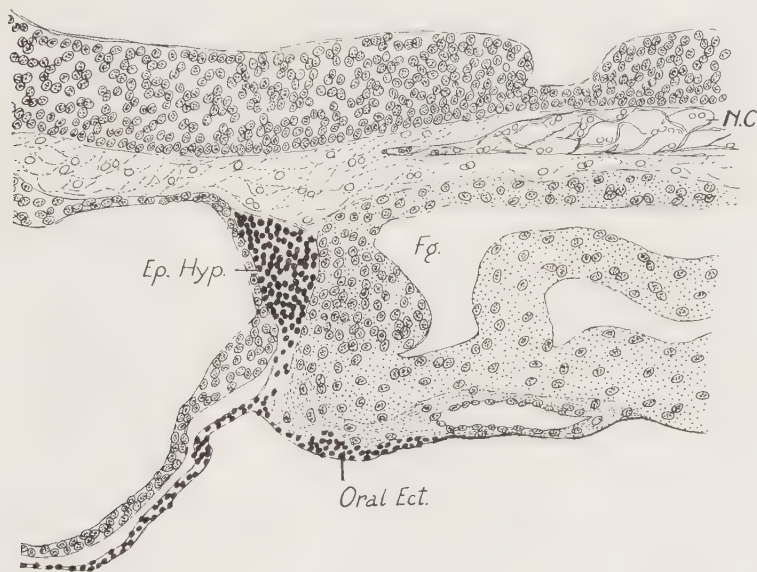


Fig. 2 Stage 22, *Cryptobranchus allengheniensis*. Camera lucida drawing of a sagittal section showing the separation of the buccal anlage of the hypophysis from the oral ectoderm. The anterior end of the section is to the left. Ep.Hyp., epithelial hypophysis; Fg., foregut; Oral Ect., oral ectoderm; N.C., notochord. $\times 100$.

the oral ectoderm by a stalk, is losing that connection in this stage (fig. 2). In developing further, it breaks away at a distance from the oral ectoderm, leaving a fairly long stalk connected with the oral epithelium. Atwell ('21) says that in *Amblystoma* this stalk "is apparently retracted into the epithelium, or else disappears. I have never noted in the *Amphibia*, a separation of the stalk close to the ectoderm

with the consequent formation of remnants, such as frequently are seen in mammals, and which may give rise to a 'pharyngeal hypophysis' or a 'parahypophysis.''' The epithelial anlage of the hypophysis is shorter and broader than in the earlier stage and tapers dorsocephalad more than it does ventrad.

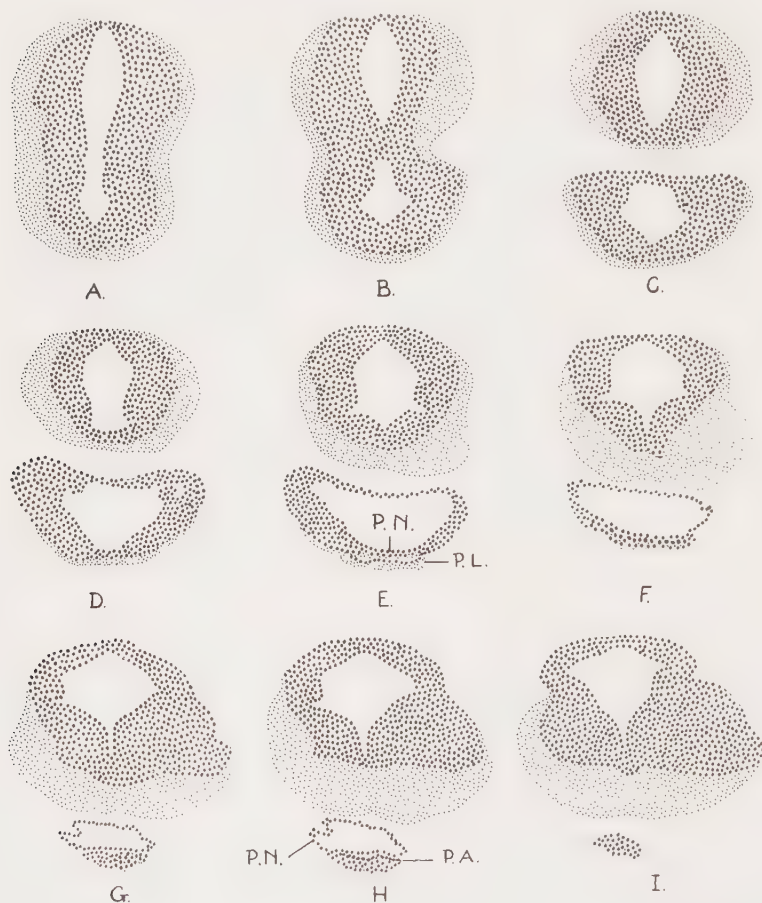


Fig. 3 Stage 23, hatching. *Cryptobranchus allegheniensis*. Camera lucida drawings of representative transverse sections of the brain and hypophysis, selected from a complete series, beginning at the anterior end. P.A., pars anterior; P.N., pars neuralis; P.L., pars lateralis. $\times 30$.

Stage 23. These specimens are 23 to 26 mm. in length, and are taken at the time of hatching. The moderate pigmentation of the two earlier stages has become more evident; the dorsal surface of the body and the sides of the tail are well pigmented. The yolk mass is now only moderately distended. The external gills are bushy. Each front limb rudiment has two digits. The pharyngeal plate has ruptured.



Fig. 4 Post-larval young of *Cryptobranchus allegheniensis*, 288 mm. body length. Camera lucida drawings of transverse sections of the hypophysis and brain. P.A., pars anterior; P.Int., pars intermedia; P.N., pars tuberalis. A, $\times 35$; B, $\times 35$; C, $\times 27.5$.

It is now possible to see an incipient, shallow pars neuralis evaginated from the tuber cinereum, and an epithelial hypophysis that shows a definite pars lateralis or future pars tuberalis (fig. 3). As yet there is no evidence of a pars intermedia. When viewed from the ventral surface (or laterally) the epithelial hypophysis is wedge-shaped, triangular in outline, the apex pointing caudad (as in a later stage represented by figs. 7 and 8).

Larval stages. In larvae of 35 to 60 mm. (3 to 9 months after hatching), no marked further development was observed

except an enlargement of the pars anterior. Except in one case, illustrated by figures 7 and 8, the pars lateralis has not yet been delimited from the pars anterior to form the definitive pars tuberalis. This case is unique in that the two lobes comprising the pars tuberalis are completely constricted off from the pars anterior. The pars intermedia is not clearly differentiated in any of these specimens. A few larvae of these ages were unpigmented and were examined for defective pars intermediae (since it is known that deficient pigmentation is frequently due to injury, necrosis or dysfunction of the pars intermedia) but no pars intermedia could be distinguished in any specimen.

The largest larva studied was 60 mm. long. It was taken late in May and its age was presumably $7\frac{1}{2}$ months after hatching, though it was longer than some 9 months larvae reared under artificial conditions. The epithelial hypophysis is still pyramidal; the pars tuberalis is still represented by a pars lateralis, and the pars intermedia is not yet distinguishable. Larvae of the second year after hatching were unobtainable. Metamorphosis occurs at the end of the second year.

Post-larval, nearly adult and adult stages. As compared with the young larvae, older individuals are more flattened dorsoventrally. The head particularly shows this flattening; it is somewhat wedge-shaped as viewed from the side. In conformity with the shape of the head, the hypophysis (figs. 4, 5 and 6) is considerably flattened dorsoventrally; this is especially evident in the pars neuralis. The post-larval, nearly-adult and adult hypophysis was essentially the same in all specimens, except for slight individual variations in the form of the pars anterior.

In actual amount of tissue, the pars anterior is the largest of the four lobes (figs. 4 and 5). Anteriorly, it lies ventral to the infundibulum (pars neuralis) and is attached to it; caudally, it underlies the pars intermedia and has no direct contact with the pars neuralis. The pars anterior is not definitely caudal to the neural lobe as in *Amblystoma* (Atwell,

'21). The fairly well-differentiated lobes comprising the pars tuberalis may be observed projecting cephalad and laterad from the pars anterior (figs. 4 and 6). They correspond to the pars lateralis of earlier stages, but the definitive pars tuberalis is partially separated from the pars anterior by a deep incisure. The pars neuralis is the most dorsal part of

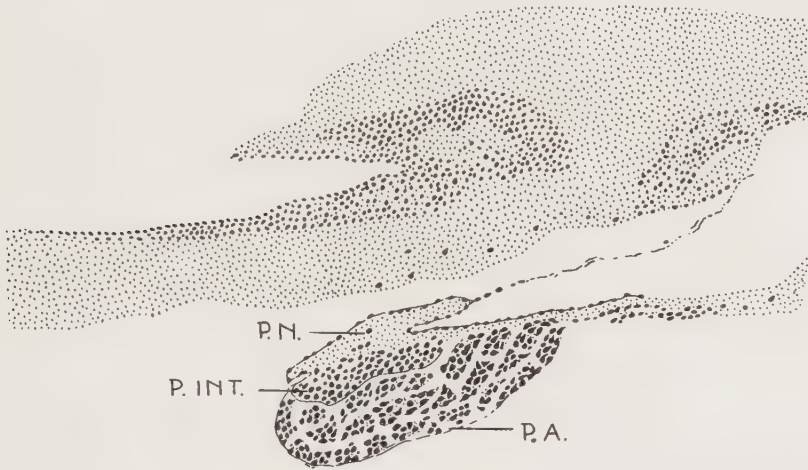


Fig. 5 Post-larval young of *Cryptobranchus allegheniensis*. Camera lucida drawing of a nearly median section through the hypophysis. P.A., pars anterior; P.Int., pars intermedia; P.N., pars neuralis. $\times 40$. The anterior end of the section is toward the right.

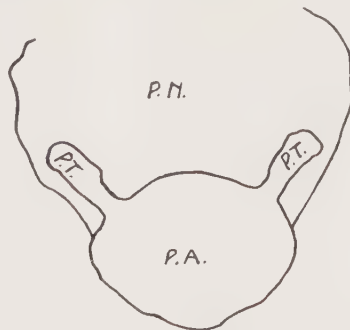


Fig. 6 Post-larval young of *Cryptobranchus allegheniensis*. Graphic reconstruction of the hypophysis to show the outline as it would appear in a ventral view. P.A., pars anterior; P.N., pars neuralis; P.T., pars tuberalis; P.Int., pars intermedia. $\times 25$.

the hypophysis; the thickness of its various portions has now become more nearly uniform. The pars intermedia lies dorsal to the caudal portion of the pars neuralis (figs. 4 and 5).

Although not included in the scope of this investigation as indicated by its title, several points of interest concerning the cytology of the hypophysis are obvious. No distinct cellular differentiation could be observed until the animals had reached a length of about 288 mm.; these were not sexually mature. In this stage (figs. 4 and 5) the pars tuberalis showed neither trabecular nor acinar arrangement of its cells. The pars intermedia appeared entirely basophilic, while the pars anterior contained not only large basophilic cells, but abundant large eosinophils. These cells of the pars anterior were arranged in cords or columns. Between these cell cords, large vascular sinuses could be seen.

DISCUSSION AND CONCLUSION

The ectodermal origin of the epithelial hypophysis (in *Amblystoma*) was definitely established by Johnston in 1910, but this contribution seems to have escaped the attention of later workers. He identified the earliest epithelial anlage of the hypophysis in embryos in the neurula stage. There is now scarcely room for doubt that the epithelial hypophysis is derived entirely from ectoderm, as stated by Atwell ('18) and Brahms ('32). The proximity of the early epithelial anlage to the entoderm, and the early disappearance of oral ectoderm from the pharyngeal plate, may readily account for the error of some authors in assuming an entodermal origin for this part of the hypophysis. Nevertheless, an ectodermal origin is clearly indicated by the character of the cells. By this, it is meant that if a careful study is made of the cells seen in the hypophysial anlage previous to patency of the pharyngeal plate, it may be readily discovered that these cells and the ectodermal cells in and around the pharyngeal plate are similar (compare the description of the appearance of stage 21 above). For *Cryptobranchus* this is shown in figure 9, and more convincingly by the direct examination

of sections. It is not very well shown in the drawings (figs. 1 and 2) since the magnification is too low to permit an accurate portrayal of the cells. In *Cryptobranchus* as in other amphibia (Atwell, '21), but not in birds or mammals, the epithelial hypophysis loses its connection with the oral ectoderm before the rupture of the pharyngeal plate. It is, therefore, obvious that the situation in *Cryptobranchus* differs from that which Valenti (1895) presents, when in disproving von Kuppfer's (1894) theory of part-ectodermal, part-entodermal origin, he states that its origin is wholly entodermal.

The notochord does not in any stage come into contact with the hypophysis, and therefore can make no contribution to it as claimed, for the amphibia, by Kuppfer (1894) and Valenti (1895).

Kingsley and Thyng ('04) came to the conclusion that the epithelial hypophysial anlage of *Amblystoma* is of ectodermal origin, but they also state that this anlage is paired in its early stages. They refer to the 'bilateral origin' of this portion of the gland. This does not agree with the results obtained by Atwell ('18, '21), nor with the results of the present investigation. The only bilateral or paired origin discernible in this species is that of the pars tuberalis, which develops out of the pars lateralis of the embryo. As suggested by Atwell ('18), an early stage in the formation of this part may have suggested a bilateral origin for the entire buccal anlage. The delayed formation of the pars lateralis in *Cryptobranchus* lends no such significance to its bilaterality.

The epithelial anlage of the hypophysis in *Amphibia* agrees with that of teleosts (Noble, '31) in arising as a solid ingrowth of ectoderm between the forebrain and the foregut, instead of presenting the appearance of an invagination as in mammals. This ingrowth loses its connection with the oral ectoderm and becomes applied to the infundibulum, a ventral diverticulum of the floor of the diencephalon. The final position of the pars anterior in *Cryptobranchus* is ventral and slightly caudal to the pars neuralis. In other *Amphibia*—

e.g., *Necturus* (Atwell, '18; Charipper, '31) and *Amblystoma* (Atwell, '21)—the pars anterior is more decidedly caudal to the pars neuralis. A further characteristic of the development of the hypophysis in *Cryptobranchus* and other amphibia, is that the part taken in its formation by the neural lobe is less prominent than it is in mammals.

In *Cryptobranchus* as in certain other Amphibia, the pars tuberalis and the pars intermedia first appear in their definitive forms soon after metamorphosis. Whether this coincidence, between a critical stage in the life history and glandular development as represented by lobe formation, has a functional significance, is uncertain. Sumi ('24) appears to think that it has. In *Cryptobranchus* the hypophysis in this stage is cytologically undifferentiated and appears incapable of glandular function.

In only one case (figs. 7 and 8), was there a separation of the pars tuberalis from the pars anterior, such as Sumi ('26) found in *Diemyctylus* and *Megalobatrachus*, or as Stendall ('13, '14) and Atwell ('18) found in the Anura.

SUMMARY

1. The hypophysis in *Cryptobranchus* consists of four parts or lobes: pars neuralis, pars anterior, pars intermedia and pars tuberalis.

2. The epithelial hypophysis is developed as a solid outgrowth from oral ectoderm, and differentiates into three lobes: pars anterior, pars intermedia and pars tuberalis.

3. There is no evidence for a bilateral origin of the anlage of the epithelial hypophysis.

4. There is no evidence for any contribution of the notochord to the formation of the gland.

5. The epithelial hypophysis loses its connection with the oral ectoderm before the rupture of the pharyngeal plate.

6. The pars anterior has the largest mass. It comes to lie ventral and only slightly caudal to the pars neuralis.

7. The pars intermedia is first seen after metamorphosis has occurred, and is differentiated from a dorsocaudal portion of the epithelial anlage, and in the nearly adult position

lies ventral to the pars neuralis and dorsal to the caudal two-thirds of the pars anterior.

8. The pars tuberalis consists of a pair of processes growing cephalad from the pars anterior (which are not joined as in reptiles, birds and mammals). In typical cases, these processes maintain a connection with the pars anterior permanently. The term pars lateralis is here applied to the anlagen of the pars tuberalis in the early stage of its development.

9. As in other Amphibia, the pars neuralis develops as an outpocketing of the floor of the diencephalon, and retains essentially this form in the adult. The distal (caudal) portion of its wall becomes thickened to form a solid mass; elsewhere its walls become thin. It contributes a relatively small part of the bulk of the entire hypophysis.

10. Differentiation of the definitive cytological characteristics of the gland begins only a few months before sexual maturity, which is attained several years after hatching.

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PLATE 1

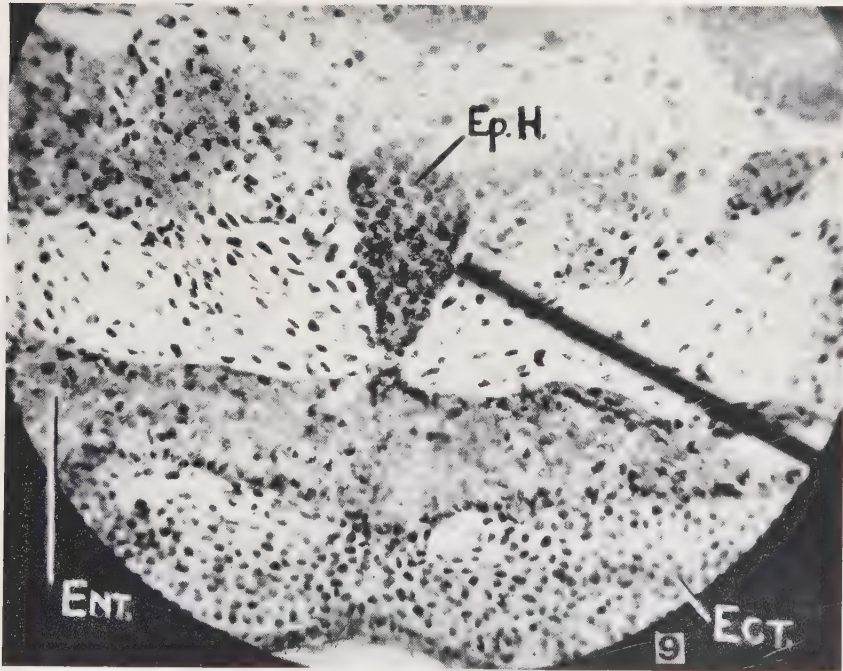
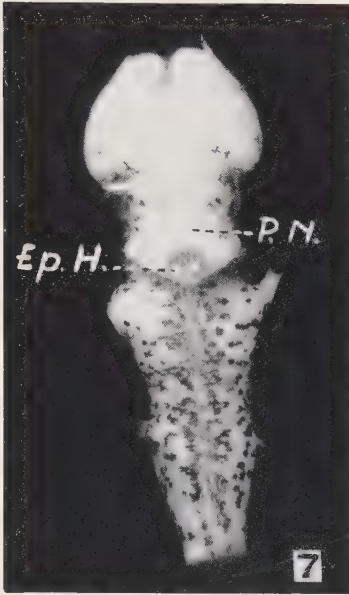
EXPLANATION OF FIGURES

Cryptobranchus allegheniensis

7 Four months after hatching; about 35 mm. body length. Photomicrograph of a ventral view of the brain. This specimen is unique in that the definitive pars tuberalis (two small round masses labeled Ep.H.) is established earlier than is usually the case, and is completely constricted off from the pars anterior. $\times 15$.

8 Same specimen in lateral view. $\times 17$.

9 Stage 21, 2 weeks before hatching. Photomicrograph of a transverse section, showing the single, ectodermal origin of the epithelial hypophysis. $\times 100$. Ep.H., epithelial hypophysis; Ect., ectoderm; Ent., entoderm of pre-oral gut.



STUDIES ON THE ORIGIN OF SHEATH CELLS AND SYMPATHETIC GANGLIA IN THE CHICK

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THREE PLATES (THIRTEEN FIGURES)

INTRODUCTION

The researches of Harrison ('04, '06, '24) have established the idea that the neural crest gives rise to sheath cells. Although this concept is widely accepted, it is not in exact accordance with Harrison's statement of the case. He demonstrated that the ventral roots of amphibian embryos developed without sheath cells subsequent to the removal of the neural crest. He noted, however, that a few cells migrated out from the neural tube into the ventral root and differentiated into sheath cells at a date later than the time of development of these cells from the neural crest. He concluded that a few sheath cells are formed from cells which migrate from the neural tube, but that the great majority of them arise from the neural crest. Detwiler ('37) has been studying the question by employing vital stains. He has demonstrated to me some preparations of amphibian embryos in which he has successfully stained the neural crest red and the neural tube blue. The sheath cells which develop subsequent to this staining technique carry the red stain, but the blue stain is confined to the spinal cord. He maintains that the earliest formed sheath cells are derived from the neural crest, but adds that the neural tube may contribute some at a later date.

Raven ('37) has applied still another technique to the problem. He transplanted tissue from *Amblystoma mexicanum* to *Triton taeniatus*. The cells of these two forms differ

sufficiently so that the host cells can be distinguished from the transplanted cells and thus the contribution of the transplanted tissue to the host can be followed. He transplanted neural crest and also the median part of the neural fold. From his data he concluded that all of the sheath cells of the amphibian originate from cells which migrate from the neural tube. Such is the status of affairs regarding the origin of sheath cells in the amphibian.

The author ('38) has demonstrated that sheath cells are abundantly present on the nerve fibers of the ventral roots of 7-day chick embryos from which the neural crest has been removed at 45 hours of incubation. This shows that the neural crest is not necessary for the production of sheath cells, but leaves one wondering about the sequence of events from the time of removal of the neural crest up to the 7-day period when sheath cells are present. In this paper the stages of development are followed in a series of experimental embryos and compared with stages of the normal embryo. Such a series also throws light upon the origin of sympathetic ganglia in the chick. Müller and Ingvar ('23) have shown that there are no sympathetic ganglia in 4-day chick embryos from which the neural crest has been removed at 48 hours of incubation. Kuntz ('22, '26), however, demonstrated sympathetic ganglia in 5-day embryos, and the author ('37) in 7-day embryos, the neural crest having been excised in each case. Although these experiments seem diametrically opposed, the following study of the stages in development following the removal of the neural crest will reveal that such is not the case.

MATERIAL AND TECHNIQUE

The embryos of the experimental series were operated upon at about 48 hours of incubation. They varied from 10 to 20 somites. The operative technique has been described in detail in a previous publication (Jones, '37). The operation consisted in cutting the ectoderm along either side of the neural tube and then cutting off a small portion of the dorsal part of the tube. The ectoderm, neural crest and dorsal part

of the neural tube were thus completely removed from the embryo. Usually the operation extended through about six consecutive segments in the most caudal part of the embryo (the future trunk region) varying more or less depending on the availability of the embryo at the operation. Hematoxylin and eosin stains were used. Part of the eggs were obtained from our colony of chickens at Loyola University School of Medicine, and part through the courtesy of Purina Mills while I was working at St. Louis University in the laboratory of Dr. Albert Kuntz to whom I wish to express my appreciation for advice and encouragement.

NORMAL DEVELOPMENT

The normal sequence of events in the formation of sheath cells and of sympathetic ganglia is shown in figures 1, 2, 3, 7. In the earliest stage (fig. 1) the ventral root has begun to sprout and a few cells are migrating along it from the spinal cord. The spinal ganglion is not shown in this photomicrograph, but its ventral extremity has reached as far as the ventral root (77-hour embryo).

A little later (fig. 2) the ventral root is more extensive and contains a large number of cells. Some of these cells have the large vesicular nucleus with darkly staining nucleolus and the dark cytoplasm characteristic of the neuroblast. Others are elongated and have the light staining cytoplasm and the dark nucleus characteristic of sheath cells. The dark cells to the right of the spinal cord belong to the dorsal root ganglion which extends down to the ventral root in adjacent sections (88-hour embryo).

At the next stage (fig. 3) the spinal ganglion is more differentiated, the ventral root and spinal nerve are free of neuroblasts but are provided with sheath cells, and the sympathetic ganglion is in a very early stage of development (91-hour embryo).

In the last stage (fig. 7) the characteristic relationships of the spinal cord, dorsal root ganglion, spinal nerve and sympathetic ganglion have been attained (101-hour embryo).

The figures presented here were chosen from the series in order to illustrate the orderly progression of development. The exact number of hours of incubation is given in each case, but it must be remembered that there is considerable variability in a group of embryos of any specific age. No doubt, the same progression could be followed in a series of embryos all at ages different than those presented here. The progressive development of the structures considered is emphasized and not the particular age at each stage except in a general comparison with the experimental series.

POST-OPERATIVE DEVELOPMENT

The experimental series consists of a group of embryos from which the neural crest and varying amounts of the dorsal portion of the neural tube have been removed for several segments in the trunk region at stages ranging from 10 to 20 somites. The dorsal roots and spinal ganglia are of course absent in this series, permitting a study of the development of the ventral roots in the absence of any influence from the neural crest derivatives.

At first (fig. 4) the ventral root closely resembles that of the normal embryo at the same age (fig. 1), although the spinal cord is open and contains fewer cells. In this figure, a few cells are present in the base of the ventral root. However, most of the roots in the operated area at this age are cell-free (77-hour embryo).

Later (fig. 5) the ventral root is quite extensive but is completely naked. The striking contrast between figure 2 (normal) and 5 (experimental) makes a description unnecessary (89-hour embryo).

At a later stage (figs. 6 and 8) the ventral root is still naked but a group of cells have migrated into the base of the nerve. In comparison with the normal embryo of the same age (fig. 3) note that the sheath cells and sympathetic ganglion are lacking (91-hour embryo).

In the next stage (figs. 10, 12) the cells in the base of the ventral root have increased in number and sheath cells have

appeared along the nerve fibers. This embryo is older than the normal one shown in figure 7, yet the sympathetic ganglion has not appeared (105-hour embryo).

At a later period (fig. 11) neuroblasts are absent from the ventral root, sheath cells are present along the nerve and the sympathetic ganglion has formed (140-hour embryo). This stage corresponds quite closely to the normal 101-hour embryo (fig. 7).

Finally (fig. 9) the picture becomes quite normal except for the absence of the spinal ganglion (180-hour embryo).

DISCUSSION

Comparing the operated with the normal series of embryos it is seen that the nerve fibers of the ventral root develop at about the same rate in both. The most conspicuous difference between the two is the absence of sheath cells on the nerve fibers in the experimental series. The nerves remain naked until around the early part of the fifth day of incubation when cells which have migrated into the ventral roots from the spinal cord differentiate into Schwann cells (fig. 10). Another striking difference is the delayed appearance of the sympathetic ganglia. In the normal series the sympathetic neuroblasts have reached their destination at 91 hours (fig. 3) but in the experimental series the sympathetic ganglia have not yet appeared at 105 hours (fig. 12). They are first visible in a 114-hour embryo. In normal embryos, cells begin migration from the neural tube about the beginning of the fourth day while in the experimental group they begin about the close of the fourth day, and then do not seem to travel as rapidly as usual since they have not proceeded into the ventral root very far at 105 hours (fig. 10) and do not form sympathetic ganglia until near the close of the fifth day. That those cells do form sympathetic ganglia has been previously demonstrated (Jones, '37).

Thus the principal consequences of extirpating the neural crest may be summarized as follows: dorsal roots and ganglia fail to develop, ventral roots remain naked until the fourth

day of incubation, and both the migration of cells from the neural tube and the formation of sympathetic ganglia is delayed for about 1 day.

The question immediately arises: Is the nakedness of the nerve fibers due to the removal of the neural crest or is it due to the delayed migration of cells from the neural tube?

In the normal embryo, as seen in figures 1 and 2, the migration of cells from the neural tube begins almost simultaneously with the sprouting of ventral root fibers. The nerve fibers are nearly obscured by numerous cells, some of which soon differentiate into sheath cells (fig. 2). Thus normally, the nerve fibers are never without their accompanying sheath cells.

In the operated embryos the nerve fibers remain naked as long as there are no cells within the ventral roots. Soon after the migration of cells into the roots, sheath cells appear (fig. 10). The differentiation of the medullary cells into sheath cells can be followed just as in the normal embryo. This eliminates the possibility that the sheath cells upon the ventral root fibers of the operated embryos might have been formed from mesenchyme cells under the influence of abnormal conditions, and confirms the prevalent idea that sheath cells are ectodermal in origin. Since the formation of sheath cells in the operated embryo is similar to their formation in normal embryos, except for the delay, it must be assumed that the nakedness of the ventral root fibers is due to the delayed migration of cells from the neural tube into the ventral root.

That sheath cells are normally formed from cells of medullary origin does not exclude the neural crest as a source of these cells. There is no evidence in the normal embryo that cells of medullary origin migrate into the neural crest. Moreover, in one experimental embryo from which the whole neural tube was removed throughout several segments, a portion of the neural crest was accidentally left in place and a spinal ganglion developed there. Although this ganglion was not connected with the spinal cord by a ventral root, nerve fibers sprouting from it were supplied with sheath cells (fig. 13).

So in the chick, evidence points to the conclusion that sheath cells arise both from the neural crest and from the neural tube. This seems altogether a good arrangement.

The delayed formation of sympathetic ganglia following the extirpation of the neural crest becomes significant when it is recalled that Müller and Ingvar ('23) did not find sympathetic ganglia in the 4-day chick which had been submitted to this operation at 48 hours of incubation. The normal 4-day embryo has sympathetic ganglia. Four-day embryos from which the neural crest has been removed do not have sympathetic ganglia. Therefore, they concluded, the neural crest is the origin of the sympathetic ganglia. The error in this logic was that the effect of the operation upon the normal developmental processes was not considered. The experimental series of embryos presented here verifies the observation of Müller and Ingvar regarding the 4-day embryo, but negates their conclusion by showing that the sympathetic ganglia appear at a later date. Thus the observations of Müller and Ingvar ('23) and of Kuntz ('22, '26) and of the author ('37) are not contradictory but are descriptions of different stages in development. I cannot, however, explain the oft-repeated and emphatic statement by Müller and Ingvar that neither in normal nor experimental embryos did they observe cells of medullary origin in the ventral roots.

Harrison's observations that in the amphibian embryo the ventral root fibers develop without sheath cells, subsequent to the removal of the neural crest, also holds true for the chick embryo. However, neither in the amphibian nor in the chick is this an adequate basis for the conclusion that the neural crest is normally the sole source of sheath cells. Harrison's conclusion that sheath cells are ectodermal in origin is confirmed by the observation that cells from the spinal cord form the sheath cells which develop on the naked ventral roots of the experimental embryos.

CONCLUSIONS

Following the removal of the neural crest in chick embryos incubated for about 45 hours, the nerve fibers of the ventral root develop at approximately the normal rate. However, the formation of the sheath cells is delayed so that for a time the ventral root fibers are naked. This delay is due to the fact that the operation upon the neural tube retards the migration of cells from the neural tube along the ventral root. This retardation postpones the development of sheath cells until approximately the beginning of the fifth day of incubation, and also delays the appearance of the sympathetic ganglia until near the close of the fifth day. These observations, together with a study of normal embryos, lead to the conclusion that the neural tube normally contributes sheath cells to the ventral root fibers. The delay in the appearance of the sympathetic ganglia explains the differing results obtained by Müller and Ingvar, who did not find sympathetic ganglia in 4-day chick embryos, and by Kuntz and the author who found sympathetic ganglia in 5- and 7-day embryos respectively, following removal of the neural crest. Until a more detailed account of the role of the neural crest in the formation of the sympathetic ganglia is made available by further research, the ventral portion of the neural tube must be considered the source of the cells which form the sympathetic ganglia.

There is no evidence that cells from the ventral part of the neural tube migrate into the neural crest to form sheath cells. There is evidence that the neural crest furnishes the sheath cells to the fibers which grow out from the spinal ganglion. In brief, the neural crest gives rise to the sheath cells of the dorsal root, the neural tube to the sheath cells of the ventral root.

That the sheath cells which develop on the naked nerve fibers are contributed by the neural tube is convincing confirmation of the observation that sheath cells are ectodermal in origin.

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PLATE 1

EXPLANATION OF FIGURES

1 Spinal cord (s.c.) and ventral root (v.) of a 77-hour chick embryo. Note cells (c.) in the base of the ventral root. $\times 657$.

2 Spinal cord (s.c.) and ventral root (v.) of 88-hour chick embryo. The cells in the ventral root have begun differentiating into sheath cells (s.) and neuroblasts (n.). $\times 567$.

3 Spinal cord (s.c.), spinal ganglion (s.g.), ventral root (v.) and sympathetic ganglion (sym.) in a 91-hour chick embryo. Note the sheath cells (s.) along the nerve trunk. $\times 485$.

4 Spinal cord (s.c.) and ventral root (v.) of 77-hour experimental chick embryo. $\times 579$.

5 Spinal cord (s.c.) and ventral root (v.) of 89-hour experimental chick embryo. Note absence of sheath cells and neuroblasts in the ventral root as compared with the normal embryo (fig. 2) at 88 hours. $\times 372$.

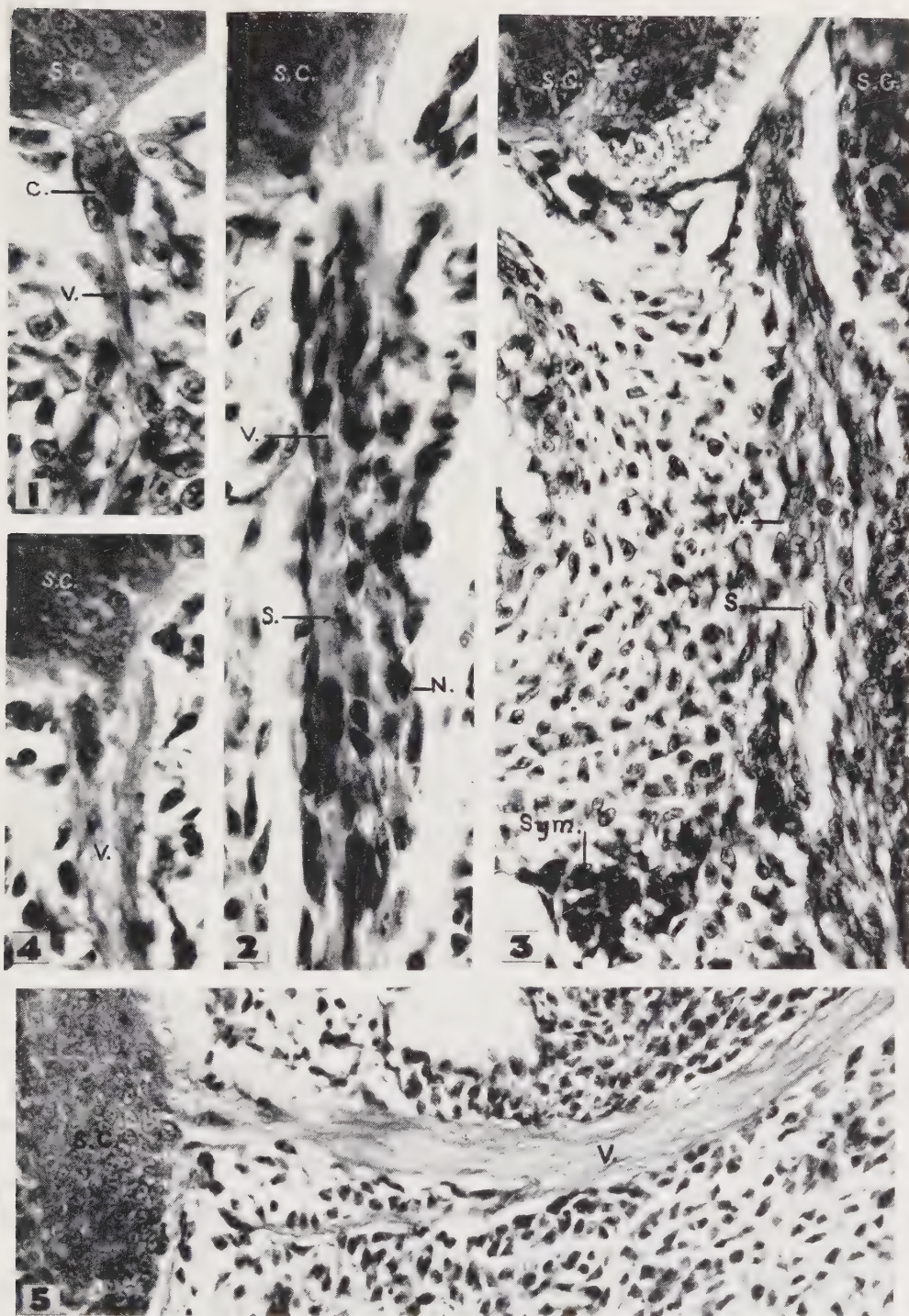


PLATE 2

EXPLANATION OF FIGURES

6 Spinal cord (s.c.) and ventral root (v.) of 91-hour experimental chick embryo. Note cells in the base of the ventral root. $\times 293$.

7 Normal 101-hour chick embryo. s.c., spinal cord; s.g., spinal ganglion; s.n., spinal nerve; sym., sympathetic ganglion; s., sheath cell. $\times 286$.

8 Higher magnification of figure 6. s.c., spinal cord; v., ventral root; c., cells in base of ventral root. $\times 668$.

9 Experimental chick embryo at 180 hours. s.c., spinal cord; v., ventral root; sym., sympathetic ganglion. $\times 90$.

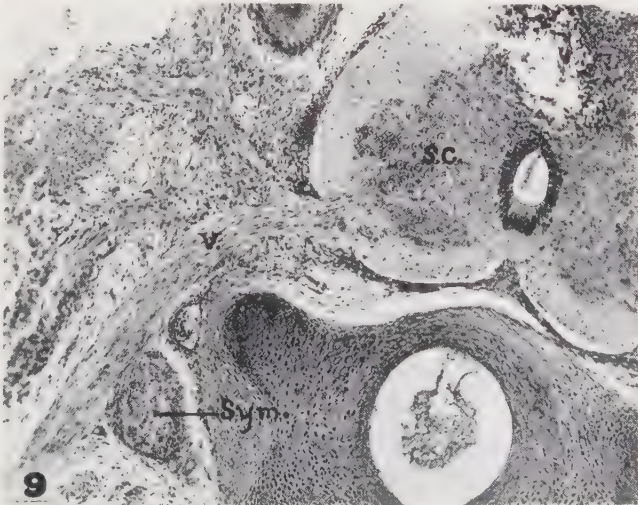
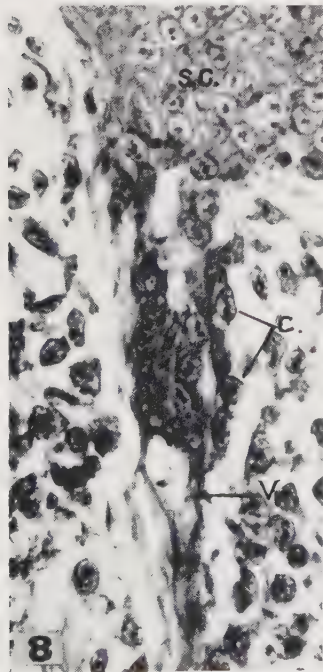
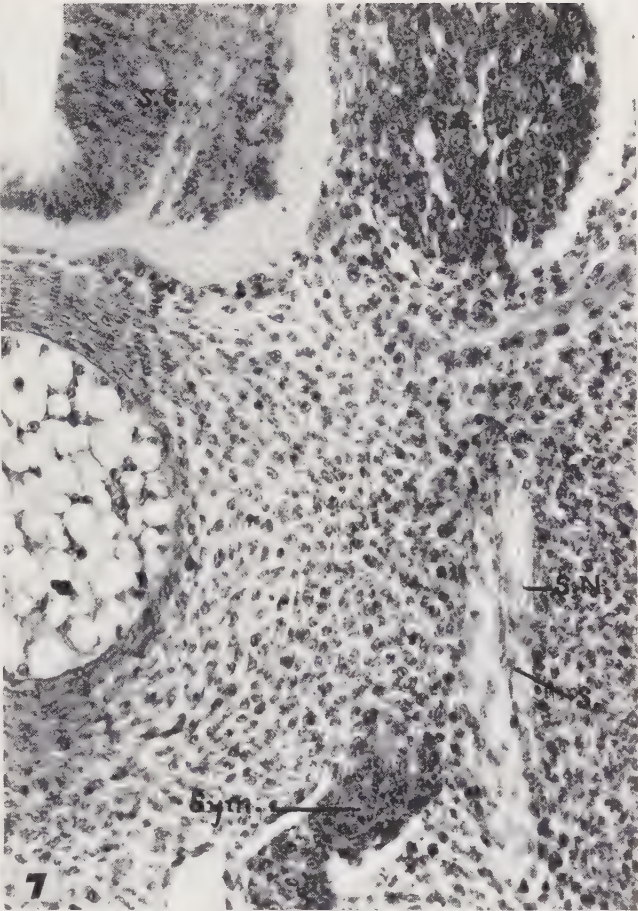
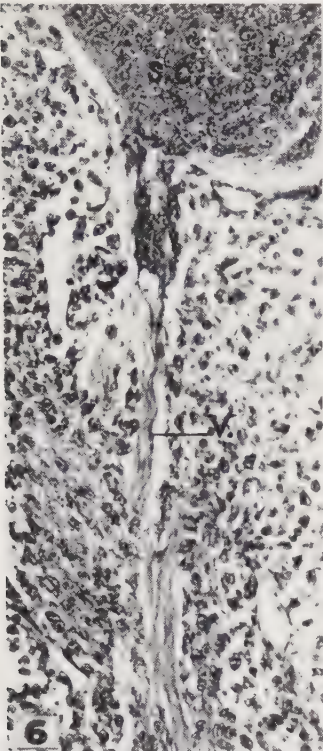


PLATE 3

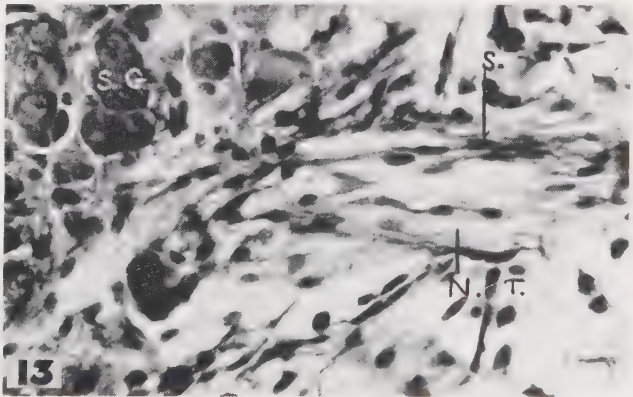
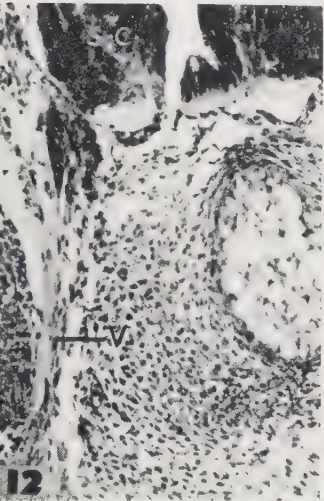
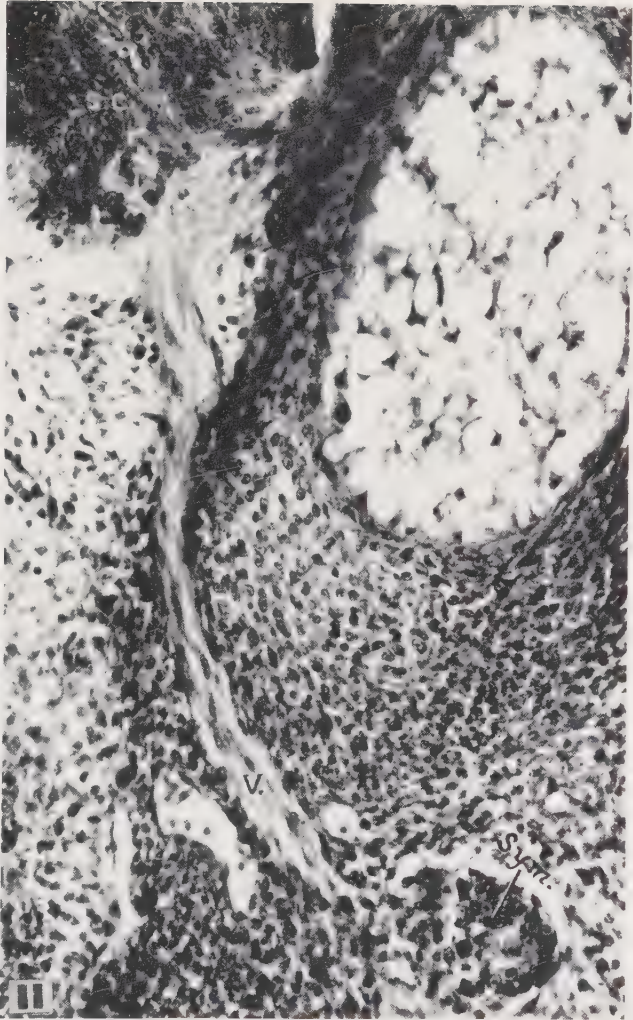
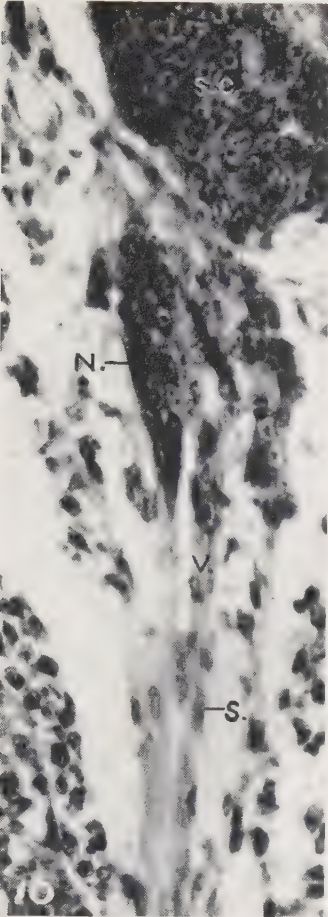
EXPLANATION OF FIGURES

10 Spinal cord (s.c.) and ventral root (v.) of 105-hour experimental chick embryo. Note sheath cells (s.) along the ventral root fibers. $\times 524$.

11 Experimental chick embryo at 140 hours. s.c., spinal cord; v., ventral root; sym., sympathetic ganglion. $\times 298$.

12 Lower power of figure 10. Note absence of sympathetic ganglion. $\times 150$.

13 Experimental chick embryo of 7 days. The spinal ganglion (s.g.) is not connected with the spinal cord in this embryo yet the nerve trunk (n.t.) sprouting from the ganglion is provided with sheath cells (s.).



AN ACIDOPHIL LINING IN BILE CAPILLARIES^{1, 2}

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ONE PLATE (NINE FIGURES)

INTRODUCTION

This investigation into the nature of bile capillary walls resulted from the observation of lines in cross sections of bile capillaries in iron hematoxylin preparations of the liver of a pregnant female domestic pig. These lines appeared as delicate continuations of the cell membranes across the region which is known as the lumen.

Many investigators in the past; McGillavry (1864), Chrzonszczewski (1866), Wyss (1866), Miura (1885), Milne ('09); have regarded the walls of bile capillaries as structures independent of the hepatic cells. Chrzonszczewski saw the wall as a clear border on indigo carmine injected bile capillaries which were isolated by teasing as well as in microscopic sections. Milne regarded the wall as a very thin flattened epithelium with branches surrounding the individual hepatic cells.

Hering (1867) considered the bile capillary wall as a condensed membrane, surface layer, or cuticle of the hepatic cells which continues into a cuticular border of the interlobular bile ducts. The latter observation was made later by Eppinger ('02) and by Rabl ('30). It was regarded by Eberth (1867) as a double contoured shining membrane secreted by the

¹ This paper is a revision of a dissertation submitted in partial fulfillment of the requirements of Niagara University for the degree of Doctor of Philosophy.

² Part of this work was demonstrated at the Pittsburgh meeting of the American Association of Anatomists, April 15, 1938. (See *Anat. Rec.*, vol. 70, Suppl. no. 3, p. 98.)

liver cells. Bloom ('25-'26) noted bile capillaries with thin double walls in a 14-mm. human embryo but did not describe them further.

Krause (1893), as interpreted by Rabl ('30), looked upon it as modified ectoplasm originating through contact with the bile and noted a transition of the wall structure into that of the cytoplasm. By a modification of the Rio Hortega silver carbonate method McIndoe ('28) also arrived at the conclusion that double contours are completely lacking in human bile capillary walls, the more intense silver impregnation of the distal portion of the wall shading off until lost in the cytoplasm. He further noted a blending of the capillary wall into the cell membranes at each side of the circular bile capillary.

Pollister ('32), in considering the bile capillary walls of *Amphiuma* prepared by the Benda method, describes and illustrates a sharply limited layer stained by crystal violet. He also notes accumulations of purple-stained material at the ends of elliptical cross sections of 2-cell capillaries which, in horizontally oriented capillaries, appear as rods. By his text and figures he clearly indicates that there is no structure distal to the sharply limited layer.

Zimmermann (1898) described terminal bars in bile capillaries. Rabl ('30) stated that these appear at times when the cell membranes and walls of the bile capillaries are very delicate and that they are lacking when the cell membranes are clearly shown and the capillary walls are thick and crusty. He regards these two types of canaliculi as interchangeable and Clara ('34) attributes the difference in appearance to different colloidal states in the wall. The present opinion of bile capillaries as stated by Rabl ('30) regards the lumen as an intercellular breach and the wall as a condensation of the cell surface which is capable of taking on different forms.

No reference to any structure distal to that surface condensation, such as was indicated by the observed lines, was found in the literature. The regularity in the position of these lines suggested that they might represent the free surface of an optically homogeneous surface modification of protoplasm

distal to that structure which is generally regarded as the bile capillary wall. With that possibility in mind the investigation was made to determine the true relationship of the lines to the walls of the bile capillaries.

MATERIALS AND METHODS

Samples of liver from a 6-month male pig that was killed by bleeding were fixed in Karpechenko's, Regaud's, Champy's and Bouin's fluids as well as in Allen's 'B-15' and a fixing agent³ which was designed in our laboratory to precipitate glycogen uniformly in hepatic cells. All material was dehydrated in 1,4 dioxane, embedded in paraffin, sectioned at 1 μ , and stained in Heidenhain's iron hematoxylin.⁴ Some slides of tissues fixed in each solution were finished without counterstain, some were counterstained with orange G in 90% alcohol, and others with fast green FCF in 100% alcohol.

A part of the material fixed in the glycogen precipitating solution was stained with Best's carmine.

Some of the Bouin-fixed pig liver was cut at 2 μ and impregnated with silver by a method described by Foley ('38) for the staining of nerve fibers.

Pieces of liver of one rat were fixed in Karpechenko's fluid and the glycogen precipitating solution immediately after death had occurred from asphyxiation in illuminating gas.

A second rat was given an intraperitoneal injection of 1% aqueous solution of chloralose shortly after a feeding of suet. When completely anesthetized a ligature was made on the common bile duct close to the liver. After allowing 3 hours for the accumulation of sufficient bile to distend the bile capillaries the common bile duct was cut in the intestinal

³Two-tenths gram of chromic acid was added to a solution of 5 ml. of glacial acetic acid and 10 ml. of formalin in 90 ml. of 1,4 dioxane. After 10 minutes the solution was decanted from the excess of chromic acid and used promptly. Material was fixed for 48 hours.

⁴Clark and Lubs phosphate buffer of pH 7.02 was substituted for distilled water in preparing the 0.5% solution of hematoxylin.

side of the ligature and the entire liver placed in Karpechenko's fluid for 10 minutes. Fixation having been initiated without releasing the pressure within the bile capillaries the liver was removed, cut into thin slices, and returned to the fixing agent. Following fixation the process of preparing microscopic slides of rat liver was the same as for similarly fixed pig material.

The last material studied was Bouin-fixed liver of the Mexican axolotl. After fixation was completed the picric acid was extracted in several changes of 70% alcohol prior to the application of 1,4 dioxane. Microscopic slides were prepared as were slides of pig and rat livers.

All observations were made with 8 $\times$ or 15 $\times$ periplanatic oculars in combination with an apochromatic oil immersion objective at its full N.A. of 1.32.

OBSERVATIONS

Domestic pig, iron hematoxylin with and without counterstain. Slides of material fixed in Champy's and Regaud's fluids offered a too hazy representation of bile capillaries to be of any assistance. Material fixed in Bouin's fluid, Allen's 'B-15,' and the glycogen precipitating agent demonstrated but vaguely the lines which were the object of this study. The specimens described below were observed in Karpechenko-fixed material in which bile capillary structure was most clearly shown.

Optically homogeneous acidophil material was generally evident distal to the hematoxylin stained structure which is known as the bile capillary wall, whether the latter was thick and crusty or very delicate and regardless of the presence of terminal bars. This inner acidophil substance was stained by orange G and by fast green FCF but was so faintly stained by iron hematoxylin that it was, unless counterstained, hardly noticeable.

In cross sections of some 2-cell canaliculi the acidophil material seemed to fill uniformly the entire region which is commonly regarded as the lumen. A median slit shaped area

in this acidophil region was more faintly stained in other bile capillaries. Many specimens demonstrated more or less clearly the lines under investigation. Each line appeared as a continuation of the cell membranes across the acidophil substance (fig. 1), joining the terminal bars wherever the latter were present.

Several capillaries were seen which were formed by surface modification of three hepatic cells. Many of these showed three lines as continuations of the cell membranes meeting in the center of the capillary (fig. 3). These lines were so delicate in some capillaries that they could not be seen with the 8 $\times$ ocular.

Other 3-cell canaliculi demonstrated a small triangular lumen although the hematoxylin stained outer structure which is known as the wall was circular in cross section (fig. 4). The limits of the lumen were defined by delicate lines. The region between these lines and the deeply stained circular structure was homogeneous and acidophil and was divided into three distinct segments, one corresponding to each contributing cell. One 4-cell capillary was observed in which the lumen was limited by four segments of acidophil material.

The maximum thickness of this acidophil lining was 0.4 μ in the 3-cell capillaries with an evident lumen. This corresponded to the greatest distance from the line to the region commonly regarded as the wall in 2-cell capillaries.

Domestic pig, silver impregnation. The cell limits in this material were indicated by a heavier impregnation than was shown by the deeper cytoplasm. In the region known as the bile capillary wall the impregnation was more dense and was sharply limited distally. Cross sections of 2-cell capillaries frequently showed a distinct dark line joining deeply stained terminal bars (fig. 2). Except for this line the region known as the lumen was homogeneous and slightly darker than the lightest parts of the cytoplasm.

Rat, normal liver, iron hematoxylin with counterstain. A single line was seen in many cross sections of 2-cell capillaries crossing acidophil material which seemed to fill the region

known as the lumen. Capillaries of this sort appeared less frequently but no less distinctly than in the pig material.

There was an evident lumen in a greater number of these capillaries. The lumen was bordered by acidophil substance which appeared as a thinner and more irregular layer than in pig material and was frequently thickest at the sides of tubule adjacent to the cell membranes.

Rat, common bile duct ligatured. The greater part of the bile capillaries were widely dilated and in these there was seldom apparent more than a trace of acidophil substance distal to the hematoxylin stained wall structure. Those capillaries which were not distended appeared as in normal rat liver.

Materials stained with Best's carmine. Comparison of pig and normal rat materials indicated a higher concentration of glycogen in the pig liver.

Mexican axolotl. The diameter of bile capillaries in this material was from two to four times that of the corresponding tubules of the domestic pig. These capillaries were sharply outlined in cross sections by a ring of uniform thickness which was deeply stained by iron hematoxylin (fig. 6). Terminal bars divided this ring into segments, each of these arcs being a part of the surface modification of one of the contributing hepatic cells.

A definite acidophil modification of the hepatic cell surface distal to the hematoxylin stained part was observed in both longitudinal and cross sections of these bile capillaries. Throughout most of its thickness of 1μ this lining consisted distally of cylindrical processes approximately 0.3μ in diameter, which were generally directed toward the center of the lumen. The nature of this inner border was best demonstrated in cross sections of bile capillaries from which a portion of the wall was eliminated by the lumen of a branch capillary (fig. 7).

The material which appeared as a sharply defined ring in cross sections was revealed in median longitudinal sections as roughly parallel rods of uniform thickness while the

processes of the brush-like border were seen extending into the lumen from each side (fig. 8). By focusing slightly above or below the median longitudinal plane, where the deeply stained portion of the wall was not included in the section, cross sections (fig. 9) of these processes were clearly shown.

The depth of the lining was not decreased in the smaller canaliculi. In them the only indication of the presence of discrete processes was found in faint radial striations such as would result from the closer spacing of the ends of these processes necessitated by the smaller capillary circumference.

Cross sections of some 3-cell capillaries were observed in which the lining, although presenting a somewhat radially striated appearance, seemed to be limited distally by lines connecting the terminal bars to outline a roughly triangular lumen (fig. 5). These capillaries bore a striking resemblance to those of the pig (fig. 4).

DISCUSSION

The lines seen in cross sections of 2-cell bile capillaries of the pig and rat were consistently more delicate than the terminal bars into which the transition was always abrupt. Many of these lines were seen in bile capillaries which lacked terminal bars and in which the region known as the wall was thick and crusty. Such observations preclude the possibility that these lines might represent side views of terminal bars at blind ends of bile capillaries, several of which were recognized; or that they might represent cell membranes beyond the capillaries.

Terminal bars in bile capillaries tilted in the plane of the terminal bars at a slight angle to the optical axis of the microscope do not appear uniformly across the capillaries as do these lines. Furthermore the translation of the image of such capillaries with a change of focus was completely lacking in cross sections of bile capillaries in which these lines were clearly observed.

Owing to the small diameter of bile capillaries of the pig and rat it was impossible to study the lines in longitudinal sections. The thickness of the sections being approximately equal to the diameter of the tubules, no properly oriented section could be obtained without including at least one terminal bar or a part of hematoxylin stained material which is regarded as the wall. In either case the structure which appears as a line in cross sections is hidden.

The uniformity in the position of the lines traversing the acidophil substance in 2-cell capillaries indicates that the latter material is not a product of secretory activity of the hepatic cells. The evidence gained from capillaries of this kind, both in iron hematoxylin stained and silver impregnated specimens, indicates that the line represents the contiguous distal surfaces of acidophil surface modifications of the opposed hepatic cells.

According to Forsgren ('29) the size of bile capillaries reaches a minimum when assimilation is at a maximum, i.e., when the cells are rich in glycogen. The high concentration of glycogen in the pig liver indicates that the frequency with which apparently closed 2-cell capillaries were observed may have been due to the functional state of the liver, the decrease in the diameter of the capillaries bringing opposed acidophil surface modifications into contact.

In no case was a lumen distinctly shown in 2-cell bile capillaries of the pig. However the more faintly acidophil shaped area sometimes viewed in their cross section suggested the presence of a lumen too small and irregular to appear open. In corresponding bile capillaries of the normal rat the apparent lumina were clearly shown and were associated with a thinner and more irregular acidophil border as well as with a lower glycogen content of the hepatic cells.

The uniform thickness of the acidophil border, its division according to the contributing cells, and the contrast between it and the deeply stained circular structure which is regarded as the wall of 3-cell capillaries lend convincing evidence that the relatively small lumen is not due to an abrupt change in

diameter of the capillaries within the depth of focus. There can be no doubt of the presence in them of a homogeneous acidophil lining which is probably a surface modification of protoplasm of the hepatic cells limited to the bile capillaries, the only free surface possessed by these cells. The double wall of bile capillaries seen by Bloom ('25-'26) in a 14-mm. human embryo may have included this lining.

The study of rat liver in which the bile capillaries were abnormally dilated indicates that the acidophil lining becomes much thinner as it is required to cover a greater area.

The deeply basophil ring and terminal bars seen in cross sections of the bile capillaries of the Mexican axolotl are probably homologous respectively to the sharply limited layer and the accumulations of purple staining material described by Pollister ('32) as the bile capillary wall of *Amphiuma*. Although he describes no wall structure distal to these it is quite possible that the acidophil brush-like lining present in the bile capillaries of the axolotl was present but overlooked in his thicker sections.

CONCLUSIONS

That specialized part of the hepatic cells which has been described by many workers as the wall of bile capillaries is an accumulation of basophil material which is located proximal to a generally thicker and acidophil structure which forms the true capillary lining.

The thickness of this distal surface layer makes the actual size of the lumen much smaller than has been supposed.

The appearance of the lining may vary with the size of bile capillaries as determined by the functional state of the liver.

In the pig, and in some capillaries of the rat and axolotl, the acidophil surface modification is clearly divided into segments corresponding to the cells of which they are specialized parts.

This lining in the bile capillaries of the Mexican axolotl has the nature of a 'brush border' inasmuch as it demonstrates discrete processes normal to the proximal portion of the wall.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

The figures consist of nine photomicrographs, the first seven of which are supplemented by enlarged drawings of the bile capillaries placed directly under the corresponding photomicrographs.

The optical system employed in making the photomicrographs consisted of a Leitz 3 lens condenser (N. A. 1.40), a Leitz apochromatic oil immersion objective (N. A. 1.32), and a Leitz 15 $\times$ periplanatic ocular. A bellows length of 20 inches provided for a magnification of $\times 2800$ and was increased to give a magnification of $\times 3000$ for figure 3.

1 One micron cross section of 2-cell bile capillary of the domestic pig, fixed in Karpechenko's fluid, stained in Heidenhain's iron hematoxylin, and counterstained in orange G. $\times 2800$. The lumen is apparently closed by the acidophil surface modifications of the hepatic cells of which the opposed distal limits appear as a single line.

2 Two micra cross section of bile capillary of domestic pig, fixed in Bouin's fluid, and impregnated with silver. Distal limits of lining appear as a single line joining terminal bars. $\times 2800$.

3 One micron cross section of 3-cell bile capillary of the domestic pig, fixed to precipitate glycogen, and stained in Heidenhain's iron hematoxylin. $\times 3000$. The lines which join the terminal bars and meet in the center indicate the distal limits of the lining.

4 One micron cross section of 3-cell bile capillary of the domestic pig, fixed in Karpechenko's fluid, stained in Heidenhain's iron hematoxylin, and counterstained in orange G. $\times 2800$. The triangular lumen is bordered by an acidophil lining which is divided into three segments corresponding to the contributing cells.

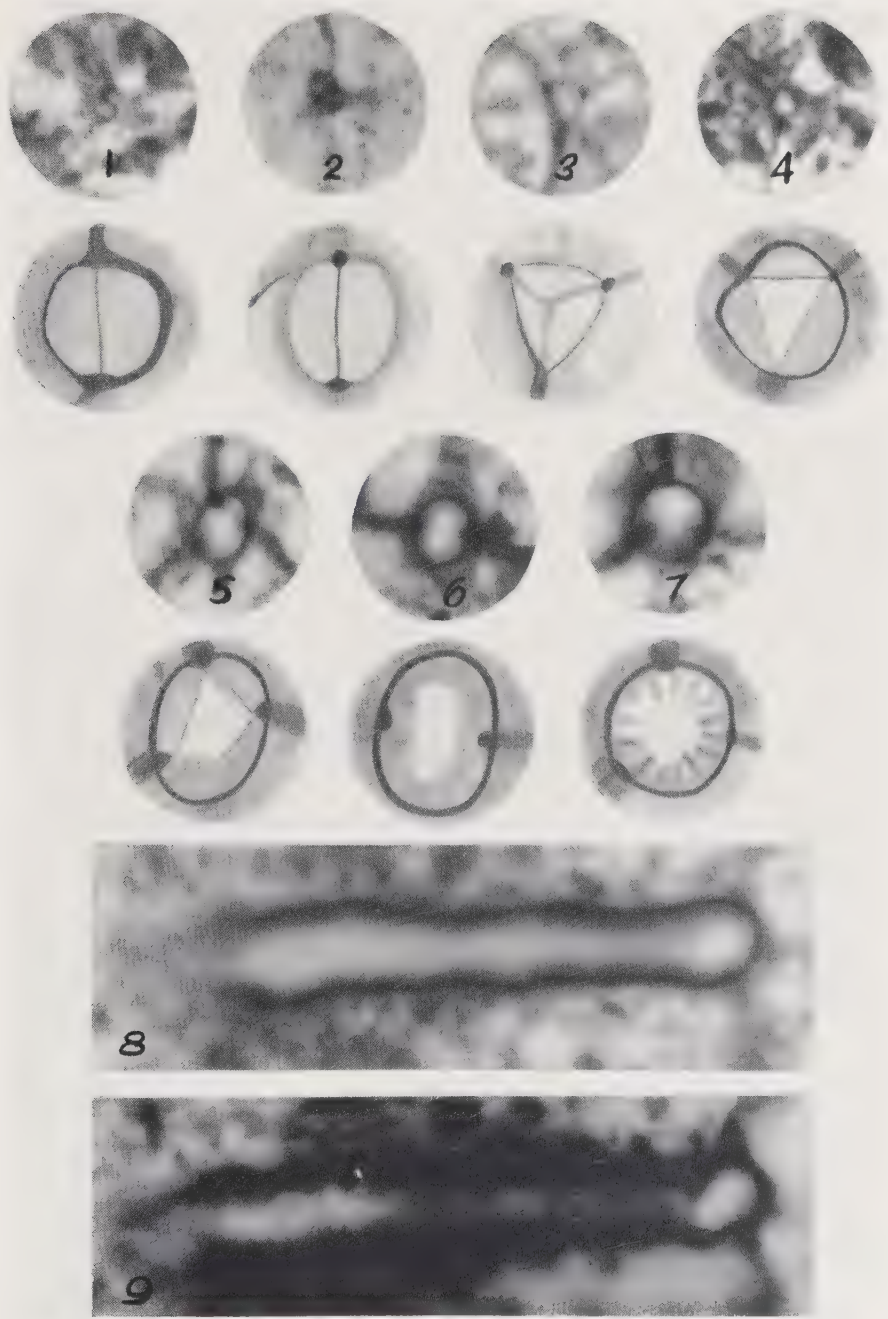
5 One micron cross section of 3-cell bile capillary of the Mexican axolotl, fixed in Bouin's fluid, stained in Heidenhain's iron hematoxylin, and counterstained with fast green FCF. $\times 2800$. Structure corresponds closely to that which is illustrated in figure 4.

6 One micron cross section of 2-cell bile capillary of the Mexican axolotl prepared as was the subject of figure 5. $\times 2800$. The acidophil lining constitutes the greater part of the wall.

7 One micron cross section of 3-cell bile capillary of the Mexican axolotl, fixed in Bouin's fluid, stained in Heidenhain's iron hematoxylin, and counterstained with orange G. $\times 2800$. In the upper side of the figure the greater part of the vertical wall is eliminated at the junction with a branch capillary aiding observation of acidophil processes.

8 One micron longitudinal section of bile capillary of the Mexican axolotl, fixed and stained as subject of figure 5. $\times 2800$. Midlevel of capillary focused to show basophil proximal portion of the wall and the distal acidophil 'brush border.'

9 Specimen shown in figure 8 with focus at slightly higher level. $\times 2800$. Cross sections of processes of 'brush border' shown at left of center.



THE SIGNIFICANCE OF THE 'KOLMER'S DROPLETS' OF THE VERTEBRATE RETINA

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The appearances known as Kolmer's droplets have been the subject of intermittent study for 30 years. It is becoming widely accepted that they are somehow concerned with the phenomena relating to rhodopsin ('visual purple'), the photo-chemical substance whose importance to vision is of the highest. Since there are great discrepancies between what is reasonable relative to rhodopsin, and what is proven fact regarding Kolmer's droplets, there is grave need at this time for an exhaustive analysis of our ideas concerning the droplets, and the bases for those ideas.

A few years ago, in a review (Walls, '34 a) in which space was at a premium, the writer categorically branded Kolmer's droplets as artefacts and stated that he had never seen them in celloidin sections stained as is recommended for their demonstration. In another footnote in the same review, he had made it clear that what he considers careful technical treatment for visual cells also involves the use of the very fixative which is likewise recommended for showing the droplets.

Because of the importance of anything that is mentioned in the same breath with rhodopsin, and because rather more has been read into the writer's above-mentioned remarks than they actually contain, he feels it to be necessary to defend his position by making the analysis suggested above.

In 1909 Kolmer described the granules or droplets which appear in the bacillary layer of retinae freshly fixed in a

bichromate-formalin-acetic mixture for at least 2 days, during the first of which, in the case of warm-blooded material, uranyl acetate and trichloroacetic acid are also ingredients of the fixative. Heidenhain's hematoxylin, destained until the rod outer segments were just blank and followed by an acid counterstain, demonstrated the droplets best. They were seen in either celloidin-paraffin or paraffin sections, clinging to the surfaces of the rod outer segments and in among the processes of the pigment epithelial cells. Their numbers were dependent upon the previous illumination of the retina, being abundant in dark-adapted, scanty or absent in light-adapted material, and varying within a single retina in accordance with the local adaptation-condition as evidenced by the position of the migratory retinal pigment.

Kolmer at first thought the droplets to be artefacts, but soon satisfied himself that they could not have been extruded from the rods. Fresh retinal smears treated with fixative and distilled water showed none on the isolated rods or pigment cell processes. Not believing in a high viscosity for the inter-bacillary lymph, Kolmer decided against their being granular precipitates of the latter and concluded that they were secretory products of the pigment epithelium.

In the frog, the administration of pilocarpine which, according to Ayres and Kühne (1878), increases the production of rhodopsin, resulted in a great increase in the droplets, which were abundant even when the retinal pigment was in its light-adapted condition. Kolmer saw and drew numbers of large droplets making their exit from the bodies of the pigment cells among the processes. The droplets apparently had as their visible precursors the myeloidin granules of the pigment cell bodies.

The droplets were seen in most classes of vertebrates. In *Lampetra fluviatilis* the evidence for their origin from the pigment cells was even better than Kolmer realized, for though he describes rows of them on the pigment-cell processes as far proximally as the outer part of the rod inner segments, he is here speaking of the cones, which with the

rods are markedly pseudo-stratified in most lampreys. The pigment cell processes do not reach even to the tips of the rod outer segments in *Lampetra* (see Walls, '35, figs. 1, 5). In teleosts the droplets were seen with difficulty unless the retinal pigment was bleached, and here there is doubt whether Kolmer was not confused by decolorized fuscin granules which of course stain readily and are of lipoid character.

In the axolotl the relation of the abundance of the droplets to dark- and light-adaptation was especially marked; so also with the frog, where it seemed scarcely comprehensible to Kolmer that they had not been described before. They were lacking in *Lacerta agilis* and *Tropidonotus* sp., whose retinae contain only cones, and were seen only on the rods in Anser. In the rat, rabbit, and lemur they were abundant among the pigment processes and upon the rods, and they were seen extrafoveally in *Alatta macaca* though not in a postmortem human neonate.

Flemming, Hermann, Zenker, sublimate-salt and sublimate-acetic fixatives all gave negative results. "Evidently no treatment other than the intensive chromation here employed will make the structures distinct and visible" (Kolmer). The droplets were concluded to be a secreted form of myeloidin made visible by swelling up in the fixative employed and preserved through the embedding process by post-chromation. Kolmer quite unwarrantedly assumed that others had not seen them because they had not chromated their material, overlooking the greater probability that the more usual fixatives fail to cause the formation of the droplets in the first place. They were not deemed artefacts despite their admitted absence from the fresh tissue, which had here assuredly been "mechanically altered from its natural state" as called for by the definition of an histological artefact in standard medical dictionaries.

It is to be noted that at this time Kolmer did no more than point out the correlation of the presence of abundant droplets with the presence of rods, retracted retinal pigment, and maximal rhodopsin; and far from concluding that the droplets

are "in some way concerned with the visual purple function of the rods" as Detwiler and Zwemer ('37) quote him, Kolmer said that only a lengthy physiological investigation would establish whether there is any genetic relationship between the droplets and the appearance of rhodopsin. This lengthy investigation has never been forthcoming.

In 1914 Kolmer succeeded in demonstrating the droplets in human neonata. His statement that the secretion-like granules from the pigment epithelium were seen in the rod outer segments is presumably a typographical error.

Detwiler ('22, '23 a) now came forward with observations on the droplets in the pure-rod retina of a gecko species. He noted that in light-adapted material, where the droplets were greatly reduced, there was a characteristic lamellar arrangement of deeply-staining granular material in the rod outer segments. In dark-adapted retinæ this 'striated material' was "greatly reduced in amount or . . . not to be seen at all." Kolmer had not noticed this difference, probably because of his habit of destaining both dark- and light-adapted sections to the point where the outer segments were clear of stain, in order to differentiate the droplets maximally.

Detwiler stressed the association of the droplets with rods, and the mutual exclusiveness of the droplets and the 'striations,' which for him was evidence enough of a relationship of the two appearances—and, in turn, reason enough to believe that the droplets originated from the rod outer segments themselves and not from the pigment epithelium as Kolmer had decided on such good grounds. The linking of the droplet-striation complex with the rhodopsin phenomenon was entirely Detwiler's also, for Kolmer had seen droplets associated with cones. True, he found none in pure-cone retinæ; but this hardly "strengthened his assumption that they are concerned in rod vision and perhaps with the appearance of visual purple," for Kolmer made no such assumption.

It was on the basis of the 'striated material' that Detwiler decided, concerning the droplets, that "their genesis from the rod, therefore, seems to be less questionable than an origin from the pigment epithelial cells." In a moment, we shall examine the validity of the assumption that the droplets and the 'striations' are in any way related to each other.

Detwiler goes on to say that "further, since they are found to be more numerous in the dark-adapted retina than in the light-adapted one, the extent of their presence is more or less in correspondence with the conditions responsible for the regeneration and the bleaching of visual purple." This statement is a little difficult to comprehend. Rhodopsin is formed inside the rod outer segment¹ and can be seen micrologically as an homogeneous coloration of the rod outer segment, both in surviving rods and in material fixed with platinic chloride. It is not formed in striations and does not pass out through the neurokeratin sheath of the outer segment in the form of droplets. It does not leave the cell at all in the ordinary sense—when light strikes it it simply ceases to be rhodopsin and breaks down into one or more other substances. There is no "similarity of behavior towards light and darkness" (Detwiler, '23a) of the droplets and rhodopsin.

If the 'striations' were more evident in dark-adapted, the droplets in light-adapted material, Detwiler's suggestion would seem far more plausible.² As a matter of fact, however, the material of the 'striations' is just as abundant in dark-adapted as in light-adapted material, though not as evident in Detwiler's procedures. The striations are the same things which Detwiler and Laurens ('21), studying the developing visual cells of *Amblystoma*, decided were probably

¹ This was not surely known until Tansley's ('33) work; but Detwiler was here suggesting that "the outer segment of the rod may be more directly concerned (than the pigment epithelium) in the production of this pigment" (than was believed at the time). This was a guess, based upon the 'striations,' but one which on quite other grounds has since proved to be correct.

² Indeed, in composing the footnote ('34a) which stimulated Detwiler and Zwemer ('37) to make their contribution, the writer erroneously stated this to be the case; but Detwiler and Zwemer do not seem to have noticed the error.

transformation-products of mitochondria." They are indeed well known to be of mitochondrial origin and the consensus of opinion, with which the writer concurs, is that they are not 'striations,' or 'discs,' but portions of a spiral or spirals. These are tightly wound in some visual cells, favoring the illusion of discs when such opaque stains as hematoxylin are used (compare Franz, '34, fig. 1). In others they are more loosely coiled (contrast figs. 75 and 76 of Tretjakoff, '16). Franz ('34) has carefully examined this matter and to the satisfaction of most retinologists has entirely disposed of the old 'Plättchenstruktur' or 'discoid' theory of outer-segment structure.

These spiral filaments are just as evident in rods which lack the power to produce rhodopsin (Walls, '34 b, figs. 4, 5) as in those which possess the ability (Walls, '35, figs. 4, 5). They are as easy to see in large cones as in small rods. It is rather too much to expect these structures, formed once and for all in the embryo, to exude through a cuticle and form an oleaginous sweat on the outside, and then regenerate, or re-form from re-absorbed droplets. Those of Kolmer's droplets which do emerge from the outer segment represent losses of lipoid ground substance, not of formed elements. Detwiler's 'striations' can have nothing to do with Kolmer's droplets, or with the elaboration of rhodopsin unless they have some other function in cones and in rhodopsin-free rods.

The differences in the conspicuousness of the 'striations' in variously illuminated material are not based upon presence-or-absence, but upon differences in stainability. Many workers, including Detwiler ('16) have noticed such differences in many of the elements of the retina. Certain nuclei

³ Kolmer ('09) speaks of seeing only traces of the 'Plättchenstruktur' of the rods, but evidently realized that it was because of the destaining, not paucity, that he did not see more of it.

In passing, it may be noted that the *Amblystoma* visual cells which Detwiler and Laurens considered fully developed were actually nowhere near the adult condition. These authors also failed to notice the 'green rods' in *Amblystoma*—a type of cell which is probably of wider occurrence in salamanders than is generally supposed.

are less chromophilic after light-adaptation, others more so, and some undergo changes in shape. Nissl flakes may appear darker and crisper after dark-adaptation, more diffuse after exposure to light.⁴ The physico-chemical basis of these differences may not be as simple as was formerly thought (Tansley, '35); but the point is that when, as has been Detwiler's practice, both light- and dark-adapted sections are attached to the same slide and stained simultaneously, staining differences will evidence themselves and may mislead one into thinking them quantitative differences. The ex-mitochondrial spirals will show as well in dark-adapted material if stained properly, for they are not 'sparse or lacking' there. Johnson ('35) and Detwiler ('32) found no precise exclusiveness of droplets and 'striations' in immature material.⁵ Johnson attributes this to the immaturity, and in the case of her own material, to the fact that it was also abnormal—that is, grafted. The grafted retinae are structurally abnormal but when, in them, rods can be found showing abundant droplets and rich 'striation' simultaneously, it is difficult to see how the two appearances could be imagined to represent the extreme stages of rhodopsin formation and destruction. The substance could not well be present and absent in the same cell at the same time. As for the 'lack of precision' in the developing rat, it would seem that before it could be dismissed as due to 'immaturity' it would be necessary to establish that there is such a thing as immaturity for rhodopsin—that upon its first embryonic appearance its molecule is different, in the direction of truly qualitative imperfection, from that in the adult.

⁴ But no one has proposed that chromatin or Nissl substance must pass in and out through membranes in order to produce these differences! Detwiler ('16, point no. 5 in summary) speaks of the chromatin and Nissl substance being reduced in amount, however, rather than rendered chromophobic or dispersed.

⁵ Johnson's figure 6 shows a fair indication of the actual spiral organization of the 'striated material.' Detwiler's claim that rhodopsin and Kolmer's droplets are simultaneously demonstrable at 11 days p.p. in the developing rat is not accepted by Tansley ('33). She could see the 'discs' at 10 to 12 days with any of her fixatives and stains (and after 3 days in vitro in an ex-5-day animal) but could be sure of rhodopsin only at 15 to 16 days either by Detwiler's technique or the Stern platonic-chloride test.

Detwiler's final statement ('22) is that "it would be premature to say definitely that they (the droplets and striations) represent a histological picture of this pigment (rhodopsin)." The statement still holds, for no evidence has since been brought forward—rather, many of Kolmer's ('25) and Johnson's ('35) observations, which Detwiler and Zwemer ('37) fail to mention, constitute strong evidence to the contrary. The history of the Kolmer's-droplet problem shows that when a few years have elapsed since one tentatively advanced a theory, one tends dangerously to think that one had proved the hypothesis by suggesting it.

In 1925 Kolmer offered additional observations, though he hardly "reinvestigated a number of vertebrate retinae" as Johnson ('35) states—indeed, it is very important to note that he did not go back over his former work. The new studies were upon *Varanus* sp., 'Hatteria,' the chimpanzee and man, and led him to conclude that the droplets were not so restricted to association with rods as he had formerly thought, but were readily producible upon the cones of some forms, as well. He stated as a generalization that if a surviving retina is fixed intravascularly with the bichromate-formalin-acetic mixture, with the acetic acid present to at least 10% concentration, droplets would be visible on the rods; and with higher concentrations of acid, also upon the cones in the higher vertebrates. His convincing photomicrograph showing the droplets on the foveal cones of the chimpanzee renders it difficult to understand how a subsequent worker could reaffirm a relationship of the droplets to rhodopsin unless he is prepared to insist that cones contain rhodopsin (but see Hecht, '34, p. 759). Since the cones of many animals show some droplets and the latter are producible on the foveal and peripheral cones of apes and man, Kolmer's own conclusion was that their production, while related to adaptation, was so related "perhaps not only to that of the rod."

It is of particular interest that Kolmer was unable to produce the droplets on the 'magnificently large cones' of

'Hatteria.' This reptile, *Sphenodon punctatum*, is not comparable with the pure-cone *Varanus*,⁶ nor a lizard at all, as stated by Johnson ('35), nor are its large visual cells cones, but rhodopsin-free rods (Walls, '34 b). Kolmer suggests that droplets might be induced in *Varanus* and *Sphenodon* if the concentration of acetic acid in the fixative were raised "too high to be unobjectionable" from the standpoint of histological technique. He used routinely a concentration of 11%, which is more than twice as great as in any other fixative in common use excepting Carnoy's fluid, which is notoriously severe upon tissues rich in lipoids.

In this 1925 paper, Kolmer fully accepts Detwiler's claim that the droplets more likely come from the visual cells than from the pigment cells. Detwiler's only evidence, it will be recalled, was the inverse relation to the striations which has been disposed of above, and the presence of droplets proximally upon rods alongside which the pigment-cell processes lay only more distally—a kind of evidence which is negated by such forms as *Lampetra* (v.s.). Kolmer, however, proceeded to obtain real evidence of the best possible kind. Realizing from his methods of obtaining droplets on cones that it was the acetic acid of the fixative which was responsible for them, Kolmer applied strong concentrations to fresh isolated rods and was able to watch the droplets emerge from the outer segment, "obviously as the result of a swelling process" and resulting in a picture similar to that seen in sections. Moreover, he found that this phenomenon had already been described by Henle in 1871.

Kolmer himself no longer speaks of the droplets as a secretion. It cannot be too strongly emphasized that they are not constantly being formed in darkness but appear only as the result of the action of the fixative. Kolmer by now obviously considered the droplets artefacts⁷ produced, as he

⁶It must be remembered that simply placing a pure-cone retina in darkness does not dark-adapt it. *Tropidonotus*, *Varanus*, *Eremias* (Detwiler, '23 b) etc. are thus actually not exceptions to the rule that Kolmer's droplets can be produced in dark-adapted retinae.

⁷His words in 1909 leave no doubt that he would have thought them artefacts even then if he had not been sure that they did not come out of the rods.

states, by swelling in acetic acid, the process being quantitatively governed by the adaptation conditions. To Kolmer, the droplets were now definable as artificial extrusions of the lipoid ground substance of the visual cell outer segment.

This reasonable view is difficult to reconcile, however, with the total abandonment of the case for the epithelial origin of droplets which Kolmer had built so well in 1909. In 1925, he readily jettisons all of this evidence, even the beautiful demonstration of the effects of pilocarpine. He does not distinguish between the droplets he saw emerging from the pigment epithelium in 1909 and those he watched being produced by the visual cells in 1925. In the absence of any repetition of the 1909 work and negative findings therefrom, Kolmer might be expected to have stated that Detwiler's suggestions and his own direct observations indicated a double origin of the droplets from the visual cells and the pigment epithelial cells, coming from one or the other in certain retinae and from both in still other cases.

This double origin the writer thinks highly probable, both from the 1909 findings which remain uncontroverted and today go unnoticed, and from the fact that the visual cells and the pigment cells are not only homologous, with a common embryonic origin, but preserve into the adult condition evidences of their kinship in an array of mutual possessions—'myeloidins,' 'lipochromes,' oil-droplets and so on which appear to be identical so far as investigation has ever shown.

Moreover, all are not agreed that the interbacillary fluid is watery, and there is reason to think that some Kolmer's droplets do represent coagula of this fluid. The writer has often found large clots in this region, so firm as to stain doubly, and which he years ago suspected, for a time, of being parasitic amebae. Johnson has described such masses of droplets in some of her cystic rosettes that it would seem that there could be little rod substance left if they all came from the latter. She explains their great numbers as due to accumulation or to the absence of some agent which would normally remove them. But if they are all to come from the

rods this explanation at once breaks down, for there can be no accumulation—Kolmer has adequately proved, for those droplets that do come from visual cells, that they are formed only during fixation, very slowly when whole fixative is added to a fresh smear, much more rapidly when strong acid alone is allowed to act.⁸ To the mind of the writer, the number of droplets in Johnson's rosettes is fair evidence that the fluid in their blind cavities is denser than normal interbacillary lymph.

The most recent contribution to this subject, that of Detwiler and Zwemer ('37), demonstrates that once the droplets have been produced by the acetic acid they can be preserved by chromation (method and duration?) through any embedding method, and suggests that formalin (concentration?) may extrude droplets as effectively, perhaps by shrinking the whole rod, as acetic acid does by swelling the ground substance within the inelastic sheath. The formalin-gelatin picture requires checking with light-adapted control material before its full meaning will be known.⁹

When however, these authors speak of "the contention of Walls that the observed droplets are artefacts due to paraffin embedding," the writer is unable to follow them, for he has said only that he has not seen them in pyroxylin sections. The reason is doubtless that he never routinely post-chromates his material,¹⁰ preferring for his purposes an unobstructed view of the visual cells (Walls, '38).

CONCLUSION

'Kolmer's droplets' of lipoid material are, then, always artefacts. They are usually producible on dark-adapted rods and sometimes on light-adapted ones, often producible from

⁸ Though Kolmer describes seeing the droplets actually emerging from the rods in smears, Detwiler and Zwemer ('37) say: "Proof, however, of an actual discharge of these droplets from the rod is still wanting."

⁹ It is possible, though unlikely, that the droplets produced by formalin are of a different nature.

¹⁰ Detwiler ('23 a) had no difficulty in seeing droplets in material fixed for only 12 to 24 hours. Apparently no special chromation was then necessary.

pigment epithelial cells, readily producible from some cones, and probably capable of being formed or imitated by coagula in interbacillary lymph. They are absent at all times from the living retina, form particularly if not solely in response to acetic acid, and have nothing to do with the formed elements of the outer segment, including the spiral filaments which Detwiler, Zwemer and Johnson call 'striated material.' In the form of Kolmer's droplets their material is not concerned with the synthesis or photic destruction of rhodopsin or with the process of vision. Their greater readiness of production in dark-adapted retinæ is only an expression of the complex physico-chemical difference known to exist between dark-adaptable retinæ in the two extreme states of illumination.

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BOOK REVIEW

DIE PERIPHERE INNERVATION. By Emil Villiger, 7th ed., revised by Eugen Ludwig, Professor of Anatomy, University of Basel. Published by Wilhelm Engelmann, Leipzig, 1938. VIII + 177 pp. 9M.

This new revision of Villiger's well-known monograph is an up-to-date compilation of the origin, course and ultimate destination of the peripheral nerves (spinal, cranial and sympathetic), together with brief summaries of their central connections. The segmental representation of the various muscles and cutaneous areas is given in considerable detail as is also the function of each muscle. The configuration and components of the various nerve plexuses are clearly presented. The book is enriched by many tables, diagrams and schemas. There are seventy-one well-chosen figures. The one showing the course of the taste fibers (fig. 39, p. 97) still retains the improbable circuit through the semilunar ganglion and sensory root of the trigeminal nerve. As Professor Ludwig admits in the accompanying explanation, there is no justification for this. It should have been eliminated 25 years ago—yet this error continues to be reproduced in a number of modern textbooks.

The so-called spinal parasympathetic system is wisely limited to a single paragraph in which the ideas of Kuré and his followers are briefly stated. The author is noncommittal.

About thirty pages are devoted to the chief sensory and motor disturbances which follow the more common lesions of the peripheral nerves and their involvement in the central nervous system.

The latest official German terminology is used throughout. This is a revision of the B.N.A. by the Nomenklatur-Kommission, headed by Prof. H. Stieve of the University of Berlin. The latest published changes appeared in the *Anatomischer Anzeiger* in 1937. To facilitate the transition to this new nomenclature the last six pages contain about 300 new terms with their older (B.N.A.) equivalents.

Due to the numerous details condensed into the small treatise, it is not possible to review it adequately in the space permitted. Other than the board binding, the material and workmanship is very good. There is no index; but on the whole it should continue to be a valuable tool to the clinician and an incentive to students of neuroanatomy.

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BOOK REVIEW

HANDBOOK OF HISTOLOGICAL AND CYTOLOGICAL TECHNIQUE. By R. R. Bensley and S. H. Bensley, Department of Anatomy, The University of Chicago. The University of Chicago Press, Chicago, Illinois, 1938. viii + 167 pp. \$2.00.

In this concise handbook the authors have emphasized principles rather than methods and therefore, unlike many works, the book is not a catalogue of techniques and their variants but an expansible manual.

Part I is devoted to descriptions of methods for the study of fresh tissues and the stressing of the importance of such a study by the Bensleys is a reiteration, in essence, of the caution sounded by Michael Foster against the "pitfalls of carmine and Canada balsam." Techniques are given for studying dead cells—maceration, smearing, touching, centrifuging, and perfusion. These are followed by methods for studying surviving tissues and cells—the preparation of blood plasma as a mounting medium, the use of supravital stains and a mention of microdissection. There is a chapter on the study of living cells—culture of whole blood, peritoneal fluid, and spleen, as well as in vivo studies on transparent animals, membranes, and organs. Mention is made of the quartz-rod illumination of organs. Aseptic surgical technique and the method for the insertion of transparent windows in living animals are described. Some space is devoted to the Altmann-Gersh freezing-drying method, its application and purpose.

In part II the various steps from the killing of the animal, excision, and fixation of the tissues to the labeling of the finished slides are taken up in sequence. The sections on the principles of staining, discussion of glands, osmic acid tests for fats, and iron reactions are noteworthy. Several methods are given for the demonstration of blood and lymphatic vessels, including special techniques devised in the Bensleys' laboratory and not yet included in other texts.

The book is filled with numerous bits of useful information, the results of long experience, which will prove invaluable to all students of technique. Such practical things are taken up as how to neutralize natural balsam, the proper sharpening of the microtome knife, the necessity for absolute cleanliness, and even suggestions for the disposal of waste paraffin and celloidin are made. It is also pointed out that dioxan may cause the impregnation of the Golgi apparatus to be lost.

Throughout the text, references are made to specific methods appearing in journals, and at the end of the book is a discriminating list of other works on technique, followed by a very complete index. The make-up in loose-leaf notebook form is practical and the book's contents should have a wide appeal.

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BOOK REVIEW

THE PRIMATE THALAMUS. By A. Earl Walker, Instructor in Neurosurgery, The University of Chicago. The University of Chicago Press, 1938. xxii + 321 pp., frontispiece. \$3.00.

Since the first monograph on the thalamus was written in 1834, by Stein at Copenhagen, this part of the brain has been the subject of many investigations, anatomical, experimental and clinical. The present monograph, chapter 1 of which gives an historical introduction, is a study, primarily, of the anatomical relationships of the thalamus by a series of carefully planned, skillfully executed and critically interpreted experiments on the brains of macaque monkeys. The macaque monkey was selected because its thalamus was considered representative of that of primates. A careful analysis in chapter 3, of the various nuclei, shows that with slight variations in topography the macaque thalamus has the subdivisions of the human thalamus. Such differences as exist can be bridged in homology with relatively little difficulty. The Nissl and Marchi techniques, supplemented to some extent by the Pal-Weigert method, were used.

A series of lesions for the study of different connections of the thalamus show a well-localized and relatively distinct projection of each ascending sensory system in the ventral nucleus, with topical localization for each. On the basis of a series of decortication experiments, the projection of different thalamic nuclei to various parts of the cerebral cortex is described. Brief comparison is made with the results of similar experiments on the chimpanzee.

A separate chapter is devoted to the geniculate bodies and their relationship to the cerebral cortex. Nearly all of it deals with the medial geniculate body, since, as the author states, little that was new was found concerning the lateral geniculate body. However, after complete unilateral occipital lobectomy, degeneration in the opposite lateral geniculate body was observed in no case.

With reference to the medial geniculate body, the author's experiments were designed to answer these questions, namely: "1) Is the projection . . . confined to the superior lip of the first temporal convolution? 2) Is it confined to the area of koniocortex? 3) Is

there a topical localization between the medial geniculate body and the cerebral cortex? If so, what is it?" He finds that a small area of the superior surface of the first temporal convolution is almost the exclusive projection area, and that this is koniocortex, i.e., granular cortex of sensory area type. There is also a topical projection of fibers from the medial geniculate body to this cortex. The probable clinical significance of this arrangement is discussed.

The volume concludes with an important chapter on the significance of the thalamus. In this discussion the thalamic nuclei are divided into three functionally distinct groups, namely, 1) the midline nuclei, regarded as related to the axial portion of the body and to visceral sensibility; 2) the nuclei receiving fibers from primary and secondary sensory neurons and projecting to the cerebral cortex; and 3) projection nuclei which do not receive fibers from different somatosensory tracts. A discussion of these various nuclei is followed by a consideration of the corticothalamic systems. A section on the clinical significance of the thalamus undertakes to correlate anatomical and experimental data with clinical knowledge of this part of the brain. The experiments are summarized in brief protocols and results are illustrated by photomicrographs and sketches. Comprehensive bibliographies are appended to each chapter. There are separate author and subject indices.

This monograph is a welcome and valuable addition to our knowledge of the thalamus. It establishes anatomical homologies on a firm experimental basis and opens the way for sound physiological and clinical interpretation. It bids fair to rank as one of the classics in neurological literature.

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EFFECT OF MUSCULAR EXERCISE ON THE FAT CONTENT OF THE LIVER

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TWO FIGURES

It is well known that fat deposition in the liver may be produced by inadequate food, intoxication with various drugs, anemia and numerous other stimuli (Best and Taylor, '37). It appeared of interest therefore, to establish whether this fat deposition is the specific result of all these agents or whether it is a part of the non-specific response of the organism to damage, as such, which expresses itself in the 'alarm reaction' (Selye, '37, '38 a, b; Karady et al., '38; Masson, '38; Leblond and Segal, '38; MacKay and Clark, '38). In order to answer this question, a series of experiments was performed on the guinea pig in which the characteristic symptoms of the alarm reaction were elicited by excessive muscular exercise, exposure to cold or subcutaneous formaldehyde injections. It was observed that although in the doses given, all these agents elicited the typical symptoms of the alarm reaction (acute involution of the lymphatic organs, gastro-intestinal ulcers, water retention in tissues, hemoconcentration, etc.) only the animals performing muscular exercise showed marked fatty infiltration of the liver; in the other groups fat deposition was only very slight if present at all. In some of the exercised guinea pigs, hemorrhagic necroses appeared in certain parts of the hepatic parenchyma (Foglia and Selye, '38).

Since it is usually more difficult to produce fatty livers in the rat than in most other laboratory animals it seemed of

interest to establish whether exercise would have the same effect in this species as it has in the guinea pig. Preliminary experiments showed this to be the case and indicated that in the rat, as well as in the guinea pig excessive muscular exercise is much more active in eliciting fat deposition in the liver than are other damaging agents. We decided, therefore, to make a detailed study of this fat deposition both from the morphological and the chemical point of view in an attempt to gain a better understanding of the mechanism by which muscular work produces this effect on the liver.

METHODS

For all the experiments reported in this communication 'hooded' black and white rats were used. These animals came from a stock inbred for many years and were raised on 'purina' fox chow.

For the histological studies, the livers were fixed in 4% formaldehyde, cut on a frozen section microtome and stained with Sudan III.

Since it is difficult to make quantitative estimations of the fat content from histological evidence only, we made chemical determinations of the total alcohol-ether soluble material in the liver. The determinations were performed as follows: 1 gm. of liver tissue, freed as far as possible from the large blood vessels, was ground up with sand in a mortar. This material was then rinsed into an Erlenmeyer flask with 35 cc. of a mixture containing 3 parts of alcohol to 1 part of ether. The flask was then heated on a hot water bath for 15 minutes. The supernatant fluid was decanted through filter paper into a 100 cc. volumetric flask. Thirty-five cubic centimeters of the alcohol-ether mixture were added to the residue in the flask and heated again for 15 minutes. This was repeated once more, after which the volumetric flask was filled up to the 100 cc. mark with the same mixture. The 100 cc. of extract were then transferred to a beaker which had previously been weighed. The solvent was evaporated under low pressure and the residue with the beaker weighed again. The weight

of the alcohol-ether soluble fraction was obtained by subtracting the weight of the empty beaker from the latter figure. The results are expressed in the tables as the per cent of wet weight of liver represented by this fraction.

While it is true that in using this method some of the liver constituents that are not fat will also be extracted, the error is very insignificant in the case of liver tissue, as indicated by control experiments in which other methods are used.

OBSERVATIONS

In our first series fifty-one 6-month-old female rats weighing 168 to 230 gm. were used. Three of these were killed as controls. Their livers showed no morphological signs of fatty infiltration, and chemical determinations showed that the average fat content was only 5.2%. Since previous experiments have shown that fasting increases fat deposition in the liver under other experimental circumstances (Selye et al., '35; Collip et al., '35) we wanted to see whether withdrawal of food would increase the fatty infiltration of the liver caused by muscular exercise. For this purpose three rats were fasted for 24 hours during which time they were forced to run for four 1-hour periods in motor driven drum cages having a diameter of 12 inches and revolving at the rate of 18 to 22 revolutions per minute. The last exercise period was started 23 hours after the beginning of the experiment and the animals were killed immediately following the 1-hour run. Three control rats treated in the same manner, but allowed food between the exercise periods, were also killed after the last run. Table 1 summarizes the results of all the experiments of this series and shows that the average fat content of the liver of rats fasted during this 24-hour period was 9.0% as compared with the average of 7.0% in the exercised but fed controls. It appears that although feeding does not completely prevent fat deposition in the liver, it decreases it to some degree.¹

¹ After this paper was ready for the press, Kaunitz and Selzer (Z. exper. Med., Bd. 103, S. 638; 1938) published a report of experiments which also indicate that excessive muscular exercise may increase the fat content of the rat liver as judged by chemical determinations.

Histological examination of the livers of these exercised rats showed marked fat deposition especially in the peripheral portions of the lobules, while the cells in the immediate vicinity of the central veins remained relatively free of fat. Figure 1 shows the appearance of a lobule of an unexercised, fasted, control rat of this series stained with Sudan III. Only two cells in this field (marked by arrows) contain lipid granules. Figure 2 shows a lobule of the liver of a similar rat forced to perform exercise during fasting. There is intense fatty infiltration especially in the peripheral parts of the lobule. The Kupffer cells, which are barely visible at this magnification are also loaded with sudanophilic gran-

TABLE 1

*Effect of muscular exercise on the fat content of the liver in female rats
6 months old*

Time	Fasted animals				Fed animals			
0 hours	10.0	9.2	7.9	Average 9.0	7.5	6.5	7.0	Average 7.0 ¹
2 hours	9.1	8.7	9.98	Average 9.3	6.9	8.5	8.3	Average 7.9
8 hours	7.0	7.7	9.0	Average 7.9	8.8	9.0	8.5	Average 8.7
24 hours	6.0	6.7	6.9	Average 6.5	7.1	8.1	6.8	Average 7.3
48 hours	5.9	4.9	5.7	Average 5.5	6.2	7.1	6.0	Average 6.4
72 hours	4.9	4.7	4.5	Average 4.7	4.3	4.7	4.8	Average 4.6

¹ All animals represented in this table except this group were fasted during the 24 hours of intermittent exercise which immediately preceded the time marked '0 hours,' when determinations were started.

ules. It should be mentioned in this connection that in many of these exercised animals, the epithelial cells of the proximal convoluted tubules of the kidney also contained numerous lipid granules.

The remaining rats were used to determine the rate at which the fat deposited under the influence of muscular exercise would disappear from the liver. For this purpose thirty rats, 6 months of age, were exercised and fasted during 24 hours in the same manner as those used for the previous experiment; then they were divided into two groups. Fifteen of them were given food and water, after the last exercise period, while the other fifteen were given water but no food. It was assumed that at the end of 24 hours of intermittent exercise

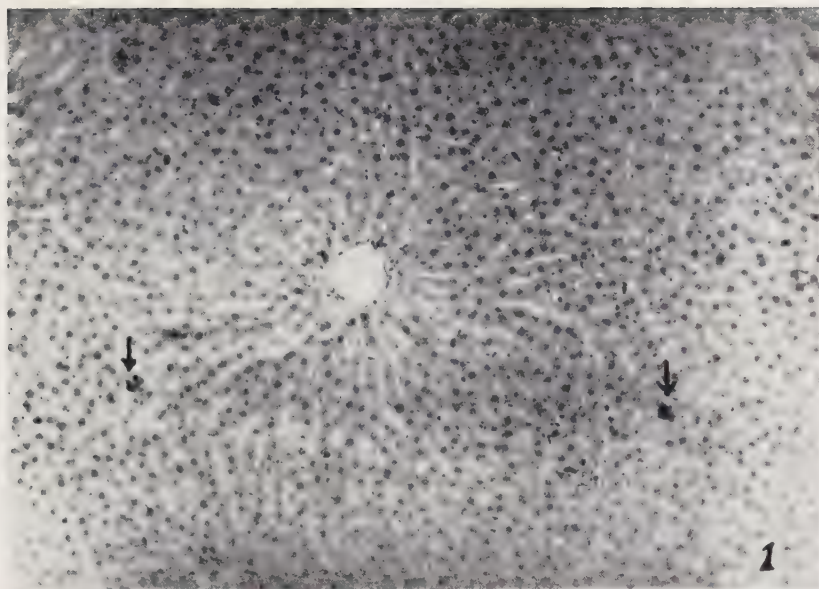


Fig.1 Frozen section of a liver lobule from an un-exercised control rat (stained with Sudan III). Only the two cells marked by arrows contain sudanophilic granules.

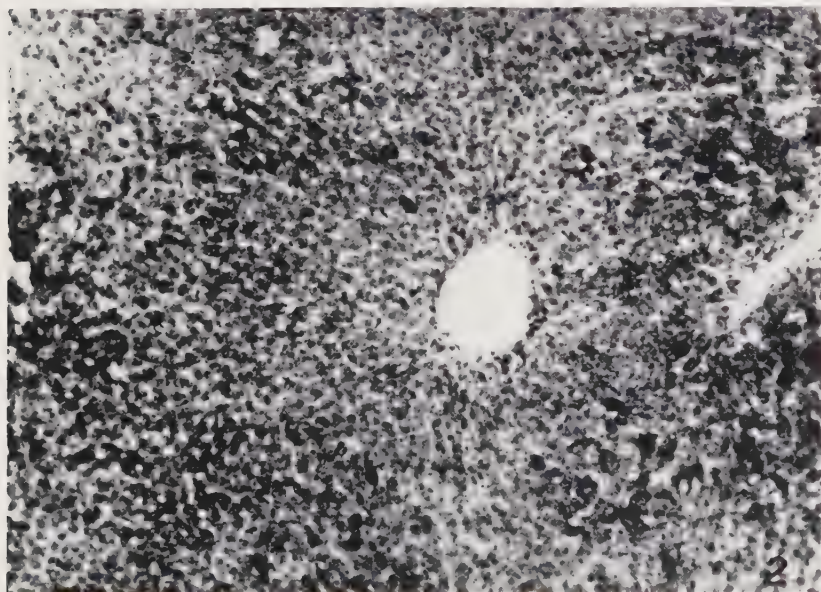


Fig.2 Frozen section of a liver lobule from a rat that performed strenuous muscular exercise (Sudan III). Note the fatty infiltration of the peripheral cells in this lobule and the sudanophilic granules in the Kupffer cells.

their livers would contain about 9% fat just as in those of fasting animals. The rate of disappearance was determined by killing three fasted and three fed rats at 2, 8, 24, 48 and 72 hours following the last exercise period. Table 1 shows that during this time there is a gradual decrease in the fat concentration of the liver, which returns to normal between the forty-eighth and the seventh-second hour. It should be emphasized that both the fasted and the fed rats represented in this table were fasted during the exercise period so as to obtain the maximum fat deposition of about 9%. The table shows that with the exception of the 2-hour group, in which for some reason the fat content is lower in the fed series, the decrease in the fat content of the liver is actually more rapid in the fasted animals. This was confirmed by the macroscopical appearance of the liver which was always more yellow in the fed group. Most recent investigators, assume that the source of the lipids in acute fatty infiltration of the liver are the fat deposits of the body. If one accepts this interpretation one might explain our findings by the assumption that if the animal receives food during the exercise period it can more easily satisfy its caloric requirements from the ingested food and does not have to draw upon its fat reserves. Consequently less fat will be discharged into the blood and available for deposition in the liver. On the other hand, if food is given after the fat is already deposited in the liver, this will decrease the necessity for drawing upon the liver fat as a source of energy.

Fasting in itself may cause fatty infiltration of the liver in certain animal species as several investigators have shown, and we can confirm this on the basis of experiments on the mouse. In the rat, however, the increase in the fat content of the liver resulting from fasting is only slight. Table 2 summarized the results which we obtained in this connection. The maximum average increase was from 5.1 to 6.3 following a 48-hour fasting period. After 72 hours, it was reduced to the initial level and stayed there even after 96 hours of fasting. If we compare the animals of table 1 with those of

table 2, we must bear in mind that the rats represented in table 1 were fasted during the 24 hours of intermittent exercise before the first group was killed so that with respect to fasting the 72-hour groups of table 1 must be compared with the 96-hour group of table 2. Such a comparison shows that 72 hours after the exercise period the fat content is actually slightly below normal, irrespective of whether the animals receive food or not.

TABLE 2

Effect of fasting on the fat content of the liver in female rats 6 months old

Fed controls	5.0	5.1	5.4	Average 5.1%
Fasted 24 hours	5.8	6.4	6.5	Average 6.2%
Fasted 48 hours	6.2	6.1	6.8	Average 6.3%
Fasted 72 hours	5.1	4.1	6.0	Average 5.0%
Fasted 96 hours	5.6	5.0	5.1	Average 5.2%

In many of our early experiments on this subject we noticed that males are less likely to develop fatty livers as a result of exercise than females. While at that time we had not as yet made quantitative determinations, sections stained with Sudan III revealed an obvious sex difference in the responsiveness of our animals. It seemed advisable, however, to confirm this observation by more quantitative methods.

TABLE 3

Effect of muscular exercise on the fat content of the liver in normal and castrate male and female rats 2 months old

Exercised	Males	6.9	6.9	6.3	5.1	7.0	5.9	Average 6.3%
Exercised	Male castrates	6.6	6.6	5.8	6.2	6.3	6.0	Average 6.2%
Exercised	Females	5.6	7.6	8.0	7.0	7.0	7.0	Average 7.0%
Exercised	Female castrates	6.5	5.6	6.5	5.9	6.6	7.1	Average 6.4%
Un-exercised controls	Males	5.0	5.3	5.5				Average 5.2%
Un-exercised controls	Male castrates	6.8	5.5	5.4				Average 5.8%
Un-exercised controls	Females	5.6	6.0	5.0				Average 5.5%
Un-exercised controls	Female castrates	6.5	6.3	5.0				Average 6.3%

Table 3 summarizes the results of chemical determinations made on a 2-month-old rats. The females in this series weighed 150 to 171 gm., the males 187 to 246 gm. We also

added one group of male and one group of female castrates in which the gonads were removed 14 days before the experiment. All these animals were fasted during the exercise period and killed immediately following the last run. They were exercised in the same manner as the rats summarized in table 1. Three normal males, three normal females and three castrate males and three castrate females were not exercised and served as controls, being killed after a 24-hour fasting period. The results shown in table 3 indicate that although the un-exercised control females and the castrate females have possibly a slightly higher liver fat content than the normal or castrate males, the difference is not very striking. Following exercise the most marked average rise occurs in the normal females, although a slight increase in fat content is also seen in the other groups. However, on the whole, the increase in fat content in this group of 2-month-old rats is much less marked than in the 6-month-old animals represented in table 1.

TABLE 4

Effect of muscular exercise on the fat content of the liver in normal and castrate male and female rats 1 year old

Male	12.0	9.0	8.5	9.5	8.0	Average	9.4%
Male castrates	5.8	9.3	7.2	7.2	7.4	Average	7.4%
Female	13.5	14.0	9.4	11.8	9.0	Average	11.5%
Female castrates	10.3	13.5	12.8	8.0	12.2	Average	11.3%

In order to obtain a clearer picture of the influence of sex on this response, we performed another experiment on animals 1 year old since we thought that possibly adult animals would react still better than those 6 months of age. Table 4 summarizes the results obtained on a group of ten males weighing 200 to 235 gm. and ten females weighing 185 to 241 gm., all of which were exercised in the same manner as the rats of the previous groups during a 24-hour fasting period and killed immediately after the last run. Half of the males and half of the females were gonadectomized 14 days prior to the experiment. It should be mentioned that although we did

not make any determinations upon unexercised adult animals for this particular experiment, numerous observations made in connection with other research subjects show that the average fat content of the liver in a normal adult male rat of our colony is about 5.5% and in an adult female 6.1%. The results of table 4 indicate that our suspicion was correct inasmuch as the adult rat responds with more marked fatty infiltration following muscular exercise than either the 2-month or 6-month age group. While this is true both of the male and the female, the fat infiltration of fat is again much more pronounced in the latter. Judged by the results shown in table 3 and table 4, castration does not seem to influence this response very much but both the male and the female castrates are perhaps slightly less responsive than the normals of the same sex.

SUMMARY

Experiments on the rat show that excessive muscular exercise causes a considerable increase in the lipid content of the liver. This increase is more marked if the animals are fasted during the period of exercise than if they are fed.

The rate of disappearance of lipids from a liver in which the fat content has been increased by muscular exercise is somewhat more rapid in animals fasted throughout the recovery period than it is in animals that receive food following the performance of exercise. It takes about 48 to 72 hours before the fat content returns to normal in such experiments.

The increase in the fat content of the liver following muscular exercise is more marked in the adult than it is in the young animal, and among animals of the same age, it is more marked in females than it is in males. This sex difference in the response seems to be relatively independent of gonadal hormones as castration does not abolish it.

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EFFECT OF OPENING THE OVARIAN BURSA ON FECUNDITY IN THE ALBINO RAT

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The encapsulation of the ovary varies between wide limits in different animal forms and has been the subject of extensive study by Arthur Robinson (1887) and by Zuckerkandl (1897). The former was the first to describe the complete sacculation of the bursa that is found in the rat and the mouse. In the human and some of the other primates the ovarian bursa is absent or nearly so. Wislocki (personal communication) writes that "a true ovarian bursa is never present in man or the anthropoid apes whereas it is present in the majority of the species of catarrhine and platyrrhine monkeys I have examined—partial encapsulation of the ovary is the rule in the platyrrhine and catarrhine monkeys." An excellent resume of the known facts about transport of the ovum is to be found in Hartman's chapter of Sex and Internal Secretions, edited by Edgar Allen ('34).

Recently Neugebauer ('35) published a report of experiments on albino rats in which he employed fifty animals that had been isolated long enough to insure they were not pregnant. He destroyed the bursae in twenty-five and in the twenty-five remaining he made an exploratory laparotomy. All of the animals were operated upon between the 10th and 15th of November and on December 15th the males were admitted. In January, pregnancy was determined by the usual signs and the animals that were found pregnant were sacrificed. His results are shown in table 1.

Neugebauer's corpora lutea counts (4.65) are very close to the general average for each ovary of the albino rat (5.20)

as reported by Long and Evans ('22). These authors also found that the albino rat has an average litter of 6.9. If we consider that an average of 10.40 ova are liberated at each ovulation, and 6.9 young produced, then 33.65% of ova do not become fertilized or if fertilized, do not develop to term. This is a larger percentage than shown in Neugebauer's control group (24.6%), but is less than half as large as the percentage loss in the experimental group (78.8%).

TABLE 1
Neugebauer's results

	TEST GROUP			CONTROL GROUP		
	Right side	Left side	Average both sides	Right side	Left side	Average both sides
Fetuses (average)	1.5	0.5	1.0	3.3	2.9	3.1
Corpora lutea (average)	5.1	4.3	4.7	4.9	4.3	4.6
Loss of ova	78.8%			24.6%		
Unilateral pregnancy	64.7%			13.9%		

In our first experiments the bursa was opened on both sides. In the control animals the bursae were exposed and not opened. The test animals were not sacrificed until 10 days after sperm were found in vaginal smears. Many of the animals passed through several cycles before sperm were found. Also, regeneration of the sac had occurred in so many instances that the results were not conclusive, though in those impregnated soon after operation the number of fetuses in each cornu was uniformly small.

The albino rat is an excellent test animal for such a determination for two reasons: first, because the ovarian bursa is complete, thereby making external migration of the ovum impossible; second, because there are two cervical canals, each opening separately into the vagina, thereby eliminating internal migration of the ovum. This makes it possible to use the ovary, tube and uterine horn on one side for the experimental test and the corresponding organs of the opposite side as a control. In this respect our experiments differ from our first series and that of Neugebauer.

The test animals were sexually mature and were having regular estrous cycles before operation, as shown by the vaginal smears. Each test animal was opened through the flank on one side, and the bursa pulled open with forceps until the ovary protruded. The animals were operated on midway between estrous onsets to permit healing before copulation. Each test animal was placed in a cage with two or three males on the day of operation and sacrificed 14 days later. The abdomen was opened and the contents of the uterine cornua

TABLE 2

No.	Side operated	Fetuses on operated side	Fetuses on unopened side	Regeneration of bursa
1	Right	1	4	Yes
2	Right	0	6	Yes
3	Right	1	4	Yes
4	Right	4	3	Yes
5	Right	0	3	Yes
6	Right	0	4	Partly
7	Right	0	3	No
8	Right	1	5	No
9	Right	1	5	No
10	Right	1	4	No
11	Right	0	2	Partly
12	Right	0	1	Yes
13	Right	3	3	Yes
14	Right	0	3	Yes
15	Right	4	4	Yes
16	Left	0	8	Yes
17	Left	1	5	Partly
18	Left	1	10	No
Total fetuses		18	77	
Average		1	4.28	

noted. The ovarian bursa was carefully examined for possible regeneration or resacculation, which can be recognized with the naked eye on picking up the bursa with small forceps. The time required for regeneration varies considerably and in some cases apparently never occurs at all. In some instances it takes place fairly early, certainly in less than 2 weeks.

Of eighteen test animals that were found to be pregnant at the end of 14 days, fifteen showed a disproportion between

the number of fetuses upon the operated and upon the unoperated side. Three of the test animals had young evenly distributed on the two sides. The results are shown in table 2.

The test average of 4.28 per horn is considerably larger than half the average number of albino rats per litter as found by Long and Evans ('22), which was 6.9 young (in 625 deliveries), or an average of 3.95 young per horn—assuming that the fetuses are in most cases evenly distributed between the two sides. This has been our observation.

It is noteworthy that in eight of the eighteen test animals there were no fetuses on the operated side (44.4%), whereas this did not occur once on the control side. Neugebauer ('35), whose work is referred to above, found that unilateral pregnancies occurred in 64.7% of his test animals.

TABLE 3
Regeneration of bursa (resacculation)

RESACCULATION	COMPLETE	NONE	PARTIAL
First series 18 test animals (present report)	10 (55.6%)	5 (27.7%)	3 (16.7%)
Second series 31 animals	18 (58.1%)	8 (25.8%)	5 (16.1%)

A complete resacculation of the bursa had occurred in ten animals (55.6%), had not occurred in five (27.7%) and had taken place partially in three (16.7%). Complete resacculation had occurred in three cases in which the young were evenly distributed on the two sides (table 3).

In another series of thirty-one animals operated in the same way regeneration of the bursa occurred as follows: complete in eighteen (58.1%); none in eight (25.8%); and partially in five (16.1%) (table 3).

The ovaries of the rat may have corpora lutea of pregnancy, of lactation and of ovulation present at one time. Since they can be differentiated satisfactorily only by means of vital dyes injected intraperitoneally (Long and Evans, '22), Neugebauer's counts seem remarkably accurate, even though the average for his experimental and test animals is slightly less than the 5.20 per ovary of Long and Evans, viz., 4.65.

The probable explanation of our three instances in which the fetuses were evenly distributed on the experimental and the control sides is that a very early regeneration of the ovarian bursa had occurred.

Although the experimental approaches made in attacking this problem by Neugebauer and by us were different, the results are essentially the same. With him we may conclude that opening the ovarian bursa in the albino rat distinctly lessens fecundity on the operated side.

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DERMATOGLYPHIC STIGMATA IN MONGOLOID IMBECILES

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In 1936 the writer presented before the American Association of Anatomists an account of the dermatoglyphics of palms and finger tips in mongoloid imbeciles ('36 a, abstract). The present note reports the findings in detail.

The literature relating to mongolism is extensive, and for the present purpose it need not be reviewed. Reference may be made to Crookshank ('31) for a general discussion of the subject and for bibliography. It is sufficient to point out here that reported observations of the mongoloid hand are restricted almost entirely to conformation and flexion furrows, though dermatoglyphics are described in several instances by Mutrux-Bornoz ('37) and Bettmann ('32) discusses the increased occurrence of palmar hypothenar and thenar patterns in individuals presenting the 'simian line' (Vierfingerfurche), thus incidentally involving mongoloids since this furrow frequently appears in them. As to hand form, it should be recalled that in most mongoloids the hand is 'lax, plump, stubby, square-palmed' (Crookshank), the digits being short, thick and somewhat tapering, the minimus often incurved. The form of the classic mongoloid hand, with its distinctive departures from the normal, should be kept in mind as the background of the dermatoglyphic peculiarities which are to be recounted.

MATERIAL AND METHODS

There are sixty mongoloid imbeciles in the series here reported, all of them inmates of Letchworth Village (Department of Mental Hygiene, State of New York). The writer

is gratefully indebted to Dr. Edward J. Humphreys, Director of Research at this institution, for making the material available, and to Dr. Charles B. Davenport for printing the subjects.

It should be noted that the sixty pairs of hands could not be used in all the observations, owing to occasional regional imperfections of the prints. Every print has been utilized to the fullest possible extent, and the omissions indicated in the results are due solely to the necessary exclusion of indecipherable regions.

Males and females are represented in nearly equal numbers, but the small size of the collection hardly justifies separate calculations of dermatoglyphic frequencies.

Since dermatoglyphics exhibit unlike trends of variation among different races the racial composition of this collection is of some importance. About half the subjects bear family names indicating that they are Jews, while the remainder have names suggesting the mixed 'European-American' stock. Data are available on the dermatoglyphic traits of both these groups (Jews—Cummins and Midlo, '27, together with unpublished material relating to a large series of German Jews; European-Americans—Cummins and Midlo, '26, also Cummins, Leche and McClure, '31). It will be understood that the following statements relating to distinctions of the mongoloid series have been checked against this normal control material, and in fact considered in the light of available literature on racial dermatoglyphics generally. The small size of the mongoloid series is disadvantageous, for dermatoglyphics are widely variable and to secure stable comparative figures the collection should be much larger. It is thought, however, that the traits disclosed may be accepted as dermatoglyphic stigmata of mongolism. The characteristics here listed neither occur invariably in all cases nor are they confined to mongolism. It is merely the extraordinary incidences of certain dermatoglyphic variants found in the sample which show that the conditioning factors for dermatoglyphic struc-

ture are placed under fairly consistent influences imposed by the state of mongolism.

Formulation of finger-tip patterns follows the familiar arch-loop-whorl classification, and the palmar features are treated by the methods of Cummins et al. ('29).

OBSERVATIONS

Finger prints. There are fifty-four individuals in the series who have completely decipherable ten-digit sets of prints. Total frequencies of the standard pattern types in these 540 digits are as follows: arches, 2.6%; ulnar loops, 75.4%; radial loops, 2.2%; whorls, 19.8%. Whorls occur with hardly more than half of the frequency which is to be expected. There is a compensating elevation of ulnar loops.

The distribution of types may be reduced to various integral expressions which are useful for group comparisons, including the index of pattern intensity of Cummins and Steggerda ('35), the whorl/loop index of Furuhashi ('27) and the arch/whorl index of Dankmeijer ('34). The mean index of pattern intensity is 11.72 ± 0.26 , reflecting the low frequency of whorls. The whorl/loop index is 25.4, a remarkably low value for the proportion of whorls to loops. The arch/whorl index is 13.1, in striking contrast to the proportionate representations of arches and whorls in normal populations having similar low frequencies of whorls (e.g., Efé pygmies of Central Africa, Dankmeijer). In assembled finger-print data drawn from diverse peoples variations in the frequencies of whorls and arches (naturally complemented by fluctuations of loops) are outstanding; there is evident a general tendency for arches to increase in frequency as whorls diminish. This tendency is conveniently expressed with the use of the arch/whorl index. If the mongoloid imbeciles fitted into the normal scheme of mutual relations between the frequencies of arches and whorls the frequency of arches would amount to several times that which occurs in this series. The departure from the normal association of arches and whorls is of no little interest as an expression of

fundamental disturbances in the pattern-conditioning mechanisms.

The percentile occurrences of pattern types on the single digits are listed in table 1. With so small a number of subjects conclusions based on their distributions must be drawn with great caution. Nevertheless, there are certain suggestive indications which are worthy of mention. Radial loops, typically chiefly concentrated on digit II, are here most abundant on digits IV-V instead. The usual superiority of the right hand in the frequency of whorls is not evident, digit IV being largely responsible for the disparity. Furthermore, plotting of figures for arches and whorls according to the dactylodialogram of Poll (published in '34, but see a more recent study,

TABLE 1
*Percentile occurrences of pattern types for each digit separately
(in fifty-four cases)*

DIGITS	I		II		III		IV		V	
	R	L	R	L	R	L	R	L	R	L
Arches	1.9	5.6	1.9	1.9	1.9		1.9	5.6	1.9	3.7
Radial loops				1.9			3.7	7.4	5.6	3.7
Ulnar loops	70.4	68.5	83.3	83.3	85.2	85.2	66.7	53.7	77.8	79.6
Whorls	27.8	25.9	14.8	13.0	13.0	14.8	27.8	33.3	14.8	13.0

'38) suggests the presence of an atypical digital distribution and symmetry.

Palmar main lines. All the main lines have been traced and formulated, but for the sake of brevity the following comments are confined to the descriptive items of major significance. The numbers of palms providing for the determinations to be mentioned are as follows: Line A—right 60, left 50; line D—right 60, left 51; line C—right 55, left 47.

The prime significance of main lines lies in the representation of the generalized directions of ridges over the palm which is afforded by lines A and D. The important characteristics of these lines may be reduced to an integral value known as the main-line index (Cummins, '36 b). The mean main-line index of the sixty right hands is 11.43 ± 0.122 ,

and that of the fifty left hands in which the necessary tracings were possible is 10.78 ± 0.172 . This result points to a significant characteristic of this mongoloid series, a prevailing tendency toward markedly transverse configurations, to a degree unmatched in any known racial group. In European-Americans the values for right and left hands respectively are 9.2 and 7.6 (Cummins, '36). If the present series were not mongoloids, but normal members of their racial stock, values closely approximating these figures would be expected. It must be noted, further, that there is a reduction of the typical bimanual inequality in this feature. Right hands in group comparison with lefts invariably show a much greater degree of transversality of configuration than lefts, as exemplified by the European-American material just cited, where the right/left ratio of the main-line index is 121. It is undoubtedly significant that the mongoloids present a diminished bimanual asymmetry, with a right/left ratio of 106.

Of the other main lines, C is of interest in a different connection. This line is the one most frequently suppressed or reduced, presenting conditions known in formulation by the symbols O, x and X. In fifty-five right hands of the mongoloid series the combined frequencies of these conditions is 7.2%, while in forty-seven left hands it is 25.5%. These frequencies, including a marked excess in left hands, are in keeping with results in normals.

Axial triradii. Axial triradii (determined in fifty-two right and forty-two left palms) show a conspicuous and significant deviation from the normal. Axial triradii located in or near the central area of the palm (in formulation, t'' , occurring either alone or in company with t , t' or both) occur in 72% of the palms, thus having about eight times the frequency of racially comparable normals. This high incidence, unknown in any reported racial group, is associated with a similarly exceptional abundance of hypothenar configurations of types L^a and A^c , with which axial triradii t'' are related.

Palmar patterns. The incidence of palmar patterns, listed in table 2, are aberrant throughout. Elevated frequencies

are noted in the hypothenar, second interdigital and third interdigital, while the thenar/first interdigital and fourth interdigital are less abundant than in normal control material.

It may be recalled (Cummins, Leche and McClure) that hypothenar, second interdigital and third interdigital patterns are characteristically more abundant in right hands of ordinary populations; thenar/first interdigitals and fourth interdigitals are more frequent in lefts. Bimanual unlike-nesses of the same order are to be noted in the mongoloid series (table 2) and in this connection it should be emphasized that the patterns displaying increased frequencies in the present series are those typically predominating in right hands, the reduced frequencies involving patterns in which

TABLE 2
Percentile occurrences of patterns and pattern vestiges in the palm

PATTERN	RIGHT		LEFT	
	Number of palms	Frequency	Number of palms	Frequency
Hypothenar	56	% 53.6	41	% 46.3
Thenar/First interdigital	57	0.0	44	6.8
Second interdigital	58	19.0	50	10.0
Third interdigital	56	89.3	47	70.2
Fourth interdigital	58	13.8	48	29.2

the excess is typically sinistral. The finding is in a sense parallel to the conditions reported for the main lines, an accentuation in both hands of dextral dermatoglyphic traits.

As to the morphological types of palmar configurations, only the hypothenar exhibits noteworthy departures from conditions in normal material. And in the instance of this configuration it must be clear that the distinction of the mongoloid series does not lie in the appearance of peculiar types of configurations, but is rather an alteration in the frequencies of types which occur in normal stocks. The distinction is an unprecedentedly high incidence of large patterns extending far distally in the palm and of the patternless configuration A^c. Their percentile frequencies follow: L^a,

38.1; S, W and Y combined, 7.2; L^c, 3.1; A^c, 20.6. The increases in L^u and A^c are particularly outstanding, since the frequencies are many times those of normal stocks (see Cummins, '35).

SUMMARY AND DISCUSSION

In this series of mongoloid idiots the dermatoglyphics of finger tips and palms present a number of characteristics markedly differing from those of racially comparable normal controls. In the finger tips there is a noteworthy reduction in the occurrence of whorls, and the expected excess of these patterns in right hands is absent. The coursing of ridges over the distal palmar region displays a heightened tendency toward transverse alignment, present in both right and left hands but with lessening of the usual dextral accentuation of this trait. Of the five palmar patterns, hypothenar, second interdigital and third interdigital patterns are increased in frequency, while thenar/first interdigital and fourth interdigital patterns are less frequent. The two groups of patterns are respectively more frequent in the right and left hands of normals, hence their behavior in mongoloids may be interpreted as an exaggerated dextral trend, in likeness to the exaggeration of transversality just mentioned. Frequencies of hypothenar types depart widely from the normal, the abundance of types L^u and A^c being especially noteworthy.

All these departures from the normal are to be regarded as stigmata of mongolism, though it must be remembered that as in other constitutional and racial expressions of dermatoglyphic variation the distinctions appear as trends characterizing the group rather than in the occurrence of traits which are diagnostic in the individual case. The distinctions of the mongoloid series are indicators of aberrant conditioning of the dermatoglyphic traits. The demonstrated aberrancies indicate that physical distinctions of mongolism exist as early as the third and fourth fetal months, the period in which the dermatoglyphics are differentiated in definitive form.

It is of interest, in the light of the views of Crookshank and others relating to racial affinities of mongoloids, to compare the dermatoglyphics of this series with the traits of normal mongolian stocks. The present observations disclose that a group of mongoloid imbeciles deviates widely from the characteristics of its own racial stock (European-Americans and Jews), and the question presenting itself is whether these deviations are approaches to the normal mongolian dermatoglyphic traits. The question is readily answered by inspection of available tabulated records (finger prints, Dankmeijer; palmar features, Steggerda, Steggerda and Lane, '36). Briefly, it may be stated that this mongoloid series differs from mongolian peoples not only in the same general directions as does the stock from which it comes, but even to larger degree since the major trends of the mongoloids represent accentuations of the very differences which distinguish the white peoples from mongolians.

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THE CELL CONTENT OF PERIPHERAL LYMPH AND ITS BEARING ON THE PROBLEM OF THE CIRCULATION OF THE LYMPHOCYTE

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TWO FIGURES

It is now well established that large numbers of lymphocytes are daily entering the blood through the thoracic duct (Winternitz, 1895; Biedl and v. Decastello, '01; Rous, '08 a, '08 b; Davis and Carlson, '09; Bunting and Huston, '21; Kindwall, '27; Haynes and Field, '31; Yoffey, '36). The question arises whether these lymphocytes which reach the blood via the lymph stream are newly formed cells, or whether they are cells which have entered the lymph from the blood stream, to which they are now returning. Some of the evidence bearing on this point has already been discussed (Yoffey, '36). Nothing is known of any possible function which such a circulation of lymphocytes might subserve, though Sjövall ('36) has suggested that there is a "synergia between reticle [reticulum] and free lymphocytes in the lymphatic tissue, in relation to this tissue as a protective system for the organism."

For the problem under consideration it is necessary to examine lymph which has not traversed a node or any organized mass of lymphoid tissue, since lymph which has made such passage contains many lymphocytes and one cannot tell whether these cells were present in the lymph before it reached the node or whether they were collected from the node during transit. In the case of lymph which has not yet traversed a node, this difficulty does not arise and we call such lymph

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peripheral² lymph, that is, lymph which has not yet passed through lymph nodes or come into contact with other collections of lymphoid tissue such as the tonsils or Peyer's patches. If peripheral lymph contains any cells, they must either have been formed by the endothelium of the lymph capillaries or have entered from the tissue spaces. There is nothing known to suggest formation of free cells on the part of the lymphatic endothelium (Clark and Clark, '32), so that any such cells in peripheral lymph must have entered from the tissue spaces, and one is thus confronted by the possibility of the origin of lymphocytes in the tissues. It is conceivable that there might be a continuous and active multiplication of lymphocytes in normal connective tissue, but there is no evidence that such multiplication occurs. Clark and Clark ('30) noted that lymphocytes in the connective tissues remained unchanged over several days. On numerous occasions they also observed the migration of lymphocytes from the blood vessels into the connective tissues (Clark and Clark, '30), and into lymphatic vessels (Clark and Clark, '37). Cells which enter the lymph from the tissue spaces are therefore cells passing from blood to lymph, and they are found in the tissues en route from one stream to the other.

MATERIAL AND METHODS

Experiments have been performed on eighteen dogs and eight cats. Lymphatics were cannulated under general (nembutal) anesthesia. From the mesenteric lymphatics lymph flowed spontaneously, from the limbs below all lymph nodes only after massage, which was performed as gently as possible. So far as could be seen, this massage did not damage the blood or lymph capillaries. Red cells were never found in large numbers in the lymph thus obtained (table 1), nor

²In addition to the designation peripheral two other terms are useful. Intermediate lymph is that which has passed through one mass of lymphoid tissue and may pass another before entering the thoracic duct. Lacteal lymph from the ileum which has been in contact with a Peyer's patch and is on the way to a mesenteric node is an example. Central lymph is that which has passed all nodes and will enter the blood stream without further change in composition.

was there any progressive increase in the white cell content (table 2). One might also expect that damage to the capillaries would result in an increased leakage of protein through their walls. However, when the protein content of massage lymph was estimated over several hours, no marked change was found (table 3). In this general connection, Clark and Clark ('37) have shown that light pressure may be sufficient to cause cells to pass from blood to lymph capillaries. That such pressure is well within the limits of normal physiological conditions is indicated by the fact that increased numbers of

TABLE 1
Red cell counts in peripheral lymph

NO. OF EXPERIMENT	ANIMAL	SOURCE OF LYMPH	ERYTHROCYTES PER CUBIC MILLIMETER
12	Dog	Left forelimb	8,000
14	Dog	Mesenteric lacteal	1,300
15	Dog	Mesenteric lacteal	1,600
16	Dog	Right hind limb	700
18	Cat	Left hind limb	5,800
		Left forelimb	13,600
19	Cat	Right hind limb	2,000
20	Cat	Right hind limb	3,500
21	Cat	Left hind limb	3,000
22	Cat	Left hind limb	300
23	Cat	Left hind limb	800
Mean			3,700

cells appear in the lymph of the dog after walking about for a short time (Drinker and Field, '33). The increase is not very great, and affects the red cells rather than the white. The fact that after massage lymph does not even show an increase in cell content, such as occurs on walking, affords additional confirmation of the view that in the present experiments the effect of massage on the capillaries was negligible.

Lymph was allowed to accumulate in the lymphatic cannula and then withdrawn by pipette. Lymph which flowed too slowly, so that sedimentation of cells might have occurred, or in which there was partial clotting, was discarded. In the

TABLE 2

Complete data from two experiments on total white cell counts in peripheral lymph.¹ All lymph collected in cannula by means of massage

NO. OF EXPERIMENT	SOURCE OF LYMPH	TIME	TOTAL WHITE CELLS
6	Left forelimb	11.00 A.M.	425
		11.10 A.M.	225
		11.20 A.M.	675
6	Right forelimb	12.25 A.M.	1,950
		12.35 A.M.	625
6	Left hind limb	3.50 P.M.	375
		4.00 P.M.	350
		4.10 P.M.	325
12	Left hind limb	10.55 A.M.	50
		11.10 A.M.	75
		11.20 A.M.	50
12	Right hind limb	2.50 P.M.	50
		3.00 P.M.	25
		3.15 P.M.	125
12	Left forelimb	6.35 P.M.	75
		6.55 P.M.	225
		7.15 P.M.	125

¹ Compare with table 5, where the same data are presented in summarized form.

TABLE 3

Absence of change in protein content of massage lymph over several hours, cannula in lymphatic vessel of right forelimb (carpal)

EXPERIMENT NO. 5		EXPERIMENT NO. 15	
Time	Protein per cent	Time	Protein per cent
11.35 A.M.	2.58	11.10 A.M.	2.10
1.35 P.M.	2.86	11.40 A.M.	2.14
2.40 P.M.	2.64	12.30 P.M.	2.36
2.50 P.M.	2.86	12.50 P.M.	2.72
3.25 P.M.	2.56	1.35 P.M.	2.50
4.25 P.M.	2.86	2.00 P.M.	2.62
		2.20 P.M.	2.66
		2.50 P.M.	2.54
		3.25 P.M.	2.68
		4.00 P.M.	2.76
		4.45 P.M.	2.66

case of each lymphatic cannulated, three consecutive white cell counts were usually made (table 2), and in eleven experiments single counts of red cells. In twenty experiments the blood lymphocytes were counted, to ascertain whether there was any constant relationship between the concentration of lymphocytes in blood and lymph.

The making of differential counts on the cells in peripheral lymph presented a distinct problem. Rous ('08 b) first pointed out the difficulty of preparing satisfactory films of lymph, and attributed it to the lack of 'body' in lymph, due presumably to the lower protein content as compared with blood. The

TABLE 4

Six differential counts on peripheral lymph (left hind limb) by the supravital technique

NO. OF EXPERI- MENT	ANIMAL	TIME	LYMPHOCYTES	MONOCYTES	MACROPHAGES	NEUTROPHILES	EOSINOPHILES	BASOPHILES	PLASMA CELLS
24	Dog	11.30 A.M.	48	15	7	19	7	2	2
		2.00 P.M.	42	20	4	24	0	6	4
25	Dog	1.50 P.M.	35	21	6	23	4	0	11
		2.30 P.M.	52	16	0	30	0	0	2
26	Cat	3.30 P.M.	79	17	2	2	1	0	0
		4.00 P.M.	37	5	4	33	20	0	0

protein content of lymph from the extremities is usually even lower than that of the thoracic duct lymph with which Rous worked, and hence the difficulty is accentuated. Most of the differential counts in the present experiments were made in the counting chamber of the hemocytometer (Sjövall, '36). Here one can readily distinguish between mononuclear and polymorphonuclear cells, and among the mononuclears it is possible to recognize the plasma cells, with small dense eccentric nucleus. One cannot distinguish, however, between lymphocyte and monocyte, and supravital preparations show (table 4) an unexpectedly large number of monocytes in peripheral lymph. Hence in most of the counts some of the

cells recorded as lymphocytes were undoubtedly monocytes, and the true lymphocyte count in peripheral lymph is somewhat lower than the figures in table 5 indicate. However, even if these monocytes are reckoned as lymphocytes, the

TABLE 5

Total white cell and lymphocyte count per cubic millimeter in peripheral lymph, lymphocyte count in thoracic duct lymph and blood

NO. OF EXPERI- MENT	PERIPHERAL LYMPH					BLOOD	THORACIC DUCT LYMPH
	No. of counts	Total white cells			Lympho- cytes	Lympho- cytes	Lympho- cytes
		Highest	Lowest	Mean			
Dogs							
1	12	625	50	270	170	1100	
2	3	225	25	150	120		
3	12	1625	75	670	380	500	
4	1	175	175	175	150	1200	
5	9	350	0	140	70		
6	8	1950	225	620	400	1700	
7	3	475	50	170	85	950	
8	9	275	0	130	50	370	
9	5	2000	800	1190	635	3750	
10	7	975	150	560	400	1900	
11	7	2500	475	1100	510	1600	12,600
12	9	225	25	90	40	900	12,050
13	2	175	100	140	75	2000	
14 ¹	7	3925	750	2300	580A 1450B	300	2,300
15	10	1800	175	810	690	800	4,400
16	6	700	100	350	140	1400	
Mean				550	280	1320	7,800
Cats							
17	3	825	300	500	460		
18	6	1375	150	520	360	5300	
19	3	625	250	425	320	3040	13,900
20	3	625	525	580	470	1100	5,100
21	3	750	50	460	370	2100	17,100
22	3	375	225	310	240	7300	
23	3	600	350	450	360	3400	
Mean				430	370	3700	12,000

¹ Two mesenteric vessels were cannulated, draining A, the upper end of the jejunum; B, the lower end of the ileum. B probably drains a Peyer's patch, and its lymph cannot therefore be regarded as peripheral. In calculating the mean lymphocyte count in peripheral lymph, B has been omitted.

count is still so low as not to make any material difference in comparisons of lymphocyte concentration in peripheral and central lymph.

DISCUSSION OF EXPERIMENTAL DATA

Lymphocyte circulation does occur, but on a small scale. Peripheral lymph almost invariably contains a few lymphocytes, and therefore some lymphocytes are always passing from blood to lymph. However, if one compares the number of lymphocytes which enter the blood stream from the lymph, with those which steadily enter the lymph from the blood the difference is so great as to make it impossible to accept the theory of the large continuous circulation of lymphocytes propounded by Sjövall ('36). The average lymphocyte count in the thoracic duct lymph of the dog (twenty-four experiments, Yoffey, '36) was 9040 per cubic millimeter. In the present experiments, where only four counts were performed, the average was 7800. The average count of lymphocytes in peripheral lymph (dog) is 280. So that for every lymphocyte which enters the lymph from the blood, about 30 lymphocytes enter the blood from the lymph.

Since, in order that the number of lymphocytes in the blood may remain constant, as many cells must enter the blood as leave it, one may say that out of every 30 lymphocytes which leave the blood 1 may be found in the peripheral lymph stream; the other 29 leave the blood and must be accounted for in some other way. These facts have been represented schematically in figure 1.

Lymphocytes are formed in the various lymphoid tissues, and may enter the blood either directly, without first of all passing into the lymph (fig. 1, A), or via the lymph stream (fig. 1, B). In B would be included lymph nodes, tonsils, Peyer's patches, solitary nodules, and the thymus if one accepts the view that it is a lymphoid organ. The most obvious example of A, lymphocytes passing directly from lymphoid tissue to blood, would be in the spleen. It has also been held that some lymphocytes may enter the blood through the

'stomata' in the post-capillary veins of lymphoid tissue described by Schulze ('25), although the existence of these stomata is open to doubt (Drinker, Field and Ward, '34).

From whatever source lymphocytes reach the blood, they do so in large numbers. Thoracic duct lymphocytes alone enter the blood in numbers sufficient to replace those already present in the blood stream at least twice a day (Yoffey, '36). Many lymphocytes must therefore either be destroyed in the blood, or else leave it for some unknown destination. Bunting

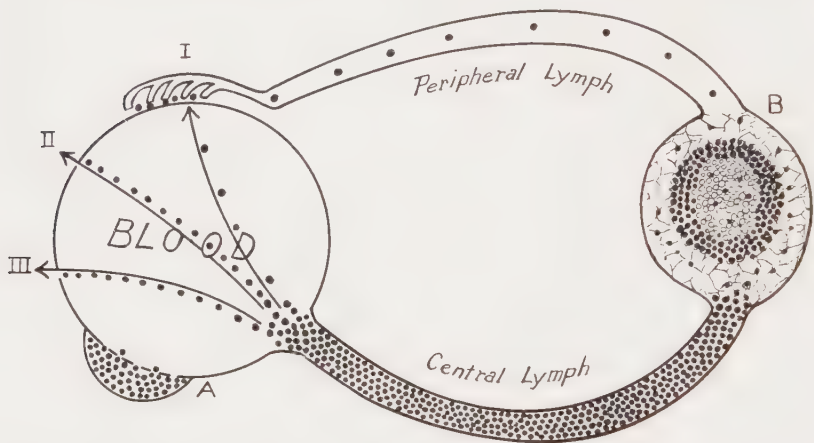


Fig. 1 Diagram illustrating some points concerning the life cycle of the lymphocyte. A, lymphoid tissues from which lymphocytes pass directly into the blood; B, lymphoid tissues from which lymphocytes first enter the lymph stream in which they are then conveyed to the blood.

and Huston ('21) adduce evidence to prove that there is no marked destruction of lymphocytes in the blood, though Wiseman ('31) is of the opinion that some of the blood lymphocytes show changes indicative of senescence. As to where the lymphocytes leave the blood, three possibilities have been suggested (fig. 1, I, II and III): I, they may pass into the connective tissues, thence into the lymphatic vessels, and back to the blood (Sjövall, '36); II, they may pass through the mucous membrane into the lumen of the gastro-intestinal tract and be lost to the animal (Bunting and Huston, '21); III,

they may be filtered out of the blood stream by the bone marrow where they are held to constitute the essential cells for blood formation.

The first of these possibilities is the one upon which our evidence bears and is in fact 'the circulation of lymphocytes.' We have found a lymphocyte content of peripheral lymph one-thirtieth that of central lymph and without any constant numerical relation to the number of lymphocytes in the blood (fig. 2). If most of the numerous lymphocytes in central

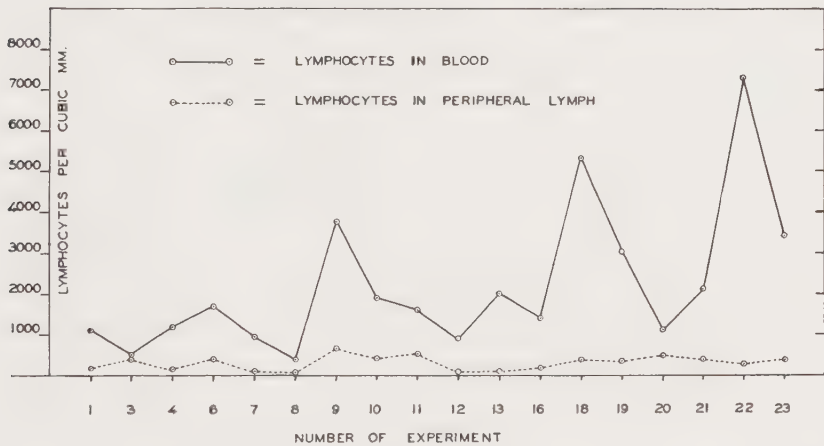


Fig. 2 Lymphocyte counts in blood and peripheral lymph in eighteen experiments. Lymphocytes in peripheral lymph do not fluctuate together with those in the blood. The lymphocyte population of peripheral lymph is maintained at a fairly constant level.

lymph were to be accounted for on the basis of circulation, that is, delivery to lymph of cells from the blood, one would have to assume that lymph nodes are capable of concentrating lymph—of reducing the volume many times by getting rid of water. If this were the fact the protein content of lymph leaving nodes should be very high as compared with lymph entering nodes. As a matter of fact many experiments have shown that lymph protein peripheral and central to nodes is identical in amount. There is thus no direct evidence for absorption of water in nodes. It may be held that fluid con-

centration is obtained by simultaneous absorption of water and lymph protein so that the concentration of the latter remains constant, but the lymphocytes per cubic millimeter of fluid increase in number. If the blood capillaries of lymph nodes are capable of large absorption of protein, they are not only unique members of the blood capillary system but at the same time display the miraculous ability to absorb protein so as to make afferent and efferent lymph precisely equal in protein content!

SUMMARY

In the peripheral lymph of sixteen dogs 110 white cell counts were made, and the mean total count was 550 per cubic millimeter, the mean lymphocyte count 280. In seven cats 24 counts were made, and the mean total white cell count was 430, the mean lymphocyte count 370. The white cell count in the lymph from any given region usually remains fairly constant, though there may be marked differences in lymph from different limbs. Peripheral lymph always contains some red cells, the mean of eleven counts being 3700.

Out of every thirty lymphocytes entering the blood through the lymph stream, twenty-nine are newly formed, one has entered the lymph from the blood. There is no rapid and extensive circulation of lymphocytes from blood to lymph.

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THE OVARY OF THE ADULT RAT

I. CHANGES IN GROWTH OF THE FOLLICLE AND IN VOLUME AND MITOTIC ACTIVITY OF THE GRANULOSA AND THECA DURING THE ESTROUS CYCLE ¹

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FOUR FIGURES

The estrous cycle and its associated changes are generally assumed to require the presence of actively functioning ovarian tissue. The fact that this tissue is subject to partial domination by the hypophysis detracts not in the least from the ultimate causal relationship which must exist between the ovary and the estrous cycle.

A reciprocal relationship between the ovary and the pituitary also seems likely (Meyer, et al., '30; Hohlweg, '34; Fevold and Hisaw, '34; Lane, '35 b, and others). This functional cooperation between the ovary and the hypophysis involves, among other things, the relation between estrogenic hormones and secretory activity by the gland. The source of estrogens in the ovary has recently been the subject of scrutiny by Mossman ('37), Corner ('38) and others, who suggest the thecae as the most likely place of origin of estrogens.

It was thought that such cyclic secretory activity as must be present in the elaboration and secretion of estrogens would disclose itself in cyclic morphologic changes, among others, by changes in mitotic activity and by volume modifications.

The colchicine technique, introduced so effectively by Allen and his group, suggested itself as a means of detecting cyclic

¹ Aided by a grant from the American Association for the Advancement of Science.

changes in mitotic activity throughout the estrous cycle. However, the ovary was found to be such an intensely metabolic organ that, after the 8-hour period during which colchicine 'collects' mitotic figures, most of the follicles exhibit granulosa layers made up almost exclusively of cells containing a blocked metaphase spindle. It became obvious that some method of studying mitotic activity was required which would indicate the division rate at a given time in the cycle. For this reason the following approach was devised.

MATERIAL AND METHOD

Twenty adult female rats of a thoroughly inbred strain were selected from a larger group. Selection was on the basis of regularity of estrous behavior. Only females showing regular cycles of 4 to 6 days were used. Regularity of estrus recurrence was established by the observation of four to eight cycles. At autopsy the rats ranged in weight from 225 to 275 gm.

The rats were killed at various stages of the cycle. Five animals were used to represent each stage. No attempt was made to obtain animals at precisely the same hour of a given phase. It was thought that a cross-sectional view would yield results of more general value than could be obtained by a more restricted method of selection. The variability of the results was naturally increased by this method of collection, but their increased validity is thought to compensate for this variation.

The rats were killed with ether, the ovaries quickly removed, freed from periovarial capsule and weighed. They were fixed in Bouin's fluid, dehydrated and cleared in dioxan and sectioned serially at 10 μ .

In studying the sections every follicle larger than 35 μ in diameter was counted and measured. The follicles were counted at the section which showed the nucleolus of the egg, since this rarely extends through two sections. In this way duplication in the count was, if not avoided, at least minimized. The follicles were classified, according to size, in

groups the extent of which was 100 μ . The diameter used in classifying the follicles was the average of the two diameters in the section showing the egg nucleolus.

Knowing the total number of follicles actually present in each of the size ranges, the volume of the granulosa, theca and antrum in an adequate, random sample of follicles in each range was determined. The formula used for calculating volume was: $V = 1/6\pi(d)(w)(1)$. In which d is the greatest over-all diameter of the follicle in the section containing the egg nucleolus; w the diameter at right angles to d , and 1 is the number of sections through which the follicle extends multiplied by the thickness of the sections.

This method was checked in several cases, by determining the area of the follicle in every section in which the follicle appeared. The sum of the areas approximated the volume determined by the above method within the limits of error.

The volume of the entire follicle, and its thecal investment was first determined. Then the volume of the follicle alone, measured to the basement membrane of the granulosa was calculated. The difference between these two measurements was the volume of the theca.

Next the volume of the antrum plus the egg was determined. In smaller follicles, in which the antrum is not yet formed, only the volume of the egg plus the zona pellucida was calculated. The antrum being, in many instances, an area of irregular outline, a certain minimum amount of 'fairing up' the outlines was required. Any error which might be introduced by this procedure would be non-directional and so may be disregarded.

The volume of the non-granulosal material, i.e., antrum and egg, was subtracted from the volume of the follicle to obtain the volume of the granulosa. The volume of the antrum, as detailed above, was determined directly.

A careful, section-by-section high-power examination was then made of the granulosa, and of the theca in each of the follicles selected for study. Mitotic figures were recorded. In follicles below 300 μ diameter, every section was so studied.

In the larger follicles enough sections were examined to total thirty, or thereabout, and the actual total number of mitotic figures was obtained, from this figure, by multiplying by the proper factor.

The 'mitotic index' which appears in the results is the number of mitotic figures to be found in a million cubic microns of either granulosa or theca tissue.

The granulosa in a total of 559 follicles of all sizes and from ovaries of animals in various stages of the estrous cycle was examined as outlined above. This represents approximately 17% of the total number of follicles contained in the ovaries of the twenty animals.

The theca was studied in 236 follicles of all sizes from ovaries of animals in various stages of the estrous cycle. The smaller number of follicles involved in the study of the theca is related to the lower level of intrinsic variability in both mitotic activity and volume changes which this tissue shows in relation to the granulosa.

Only those follicles which showed no obvious evidences of atresia were studied in this work. At a later time we hope to present a similar study on follicular atresia in the adult rat.

RESULTS

Table 1 presents the average distribution of follicles according to size throughout the estrous cycle. It will be noted

TABLE 1
Average distribution of follicles according to size throughout the estrous cycle

FOLLICLE DIAMETER	DIESTRUS		PROESTRUS		ESTRUS		METESTRUS	
	No.	% ¹	No.	%	No.	%	No.	%
35-100	130	61.3	72	53.3	63	50.0	89	56.4
101-200	55	26.0	43	31.8	41	32.5	48	30.4
201-300	12	5.7	11	8.1	11	8.7	10	6.3
301-400	7	3.3	2	1.5	5	3.9	6	3.8
401-500	4	1.9	1	0.7	1	0.8	5	3.1
501-600	3	1.4	3	2.2	2	1.6	0	
601-700	1	0.4	3	2.2	3	2.5	0	
Average								
Total	212				126		158	

¹ The percentage of the total follicle content which falls in a given size range.

that over 50% of the total content of follicles on the average, are smaller than $100\ \mu$ in diameter. This is particularly true of diestrus when 61.3% of the total follicles are below $100\ \mu$ in diameter. Indeed this can be carried still further; in all stages of the cycle over 90% of the total follicle content is below $300\ \mu$ in diameter. If all the follicles larger than $300\ \mu$ be lumped together, we arrive at the following figures: diestrus 7%, proestrus 7.6%, estrus 8.8% and metestrus 6.9%. In ovaries from animals in all stages of the cycle except metestrus, when they have been eliminated by ovulation or atresia, it is possible to find follicles between 600 and $700\ \mu$ in diameter. These follicles increase only slightly in number during proestrus and estrus.

In figure 1 is shown graphically the relationship between granulosa, theca and antrum in the growing follicle. Data for the preparation of this curve were lumped for all stages of the cycle for it was found that cyclic differences did not distort the general trend of the curves. It is to be noted that the theca, antrum and granulosa show very slow increases until the follicle reaches $200\ \mu$ in diameter, when the granulosa begins to grow more rapidly in volume than the theca. The beginning of augmented increase in volume of the granulosa may be correlated with increased mitotic activity of the granulosa in follicles of from 200 to $300\ \mu$ diameter in all stages of the cycle (table 2 and fig. 2). From this time on to the time of ovulation the granulosa shows steady increases in volume.

The theca (fig. 2 and table 3) also shows increased mitotic activity in follicles from 200 to $300\ \mu$ in diameter, which may help to account for the more rapid increase in volume occurring in this size range. Thereafter there is a steady increase in volume of this follicular component which continues to ovulation. The volume of the theca is always less than that of the granulosa and its volume changes tend to parallel those exhibited by the granulosa.

The antrum seems to exhibit three definite stages in its growth. Until the follicle has attained a size of approxi-

mately 300 μ , the increase in volume is slight. From 300 to 600 μ , the growth tends to be steady but more rapid than previously, and from 600 to 700 μ the increase in volume of the antrum is more rapid than at any other time in its development.

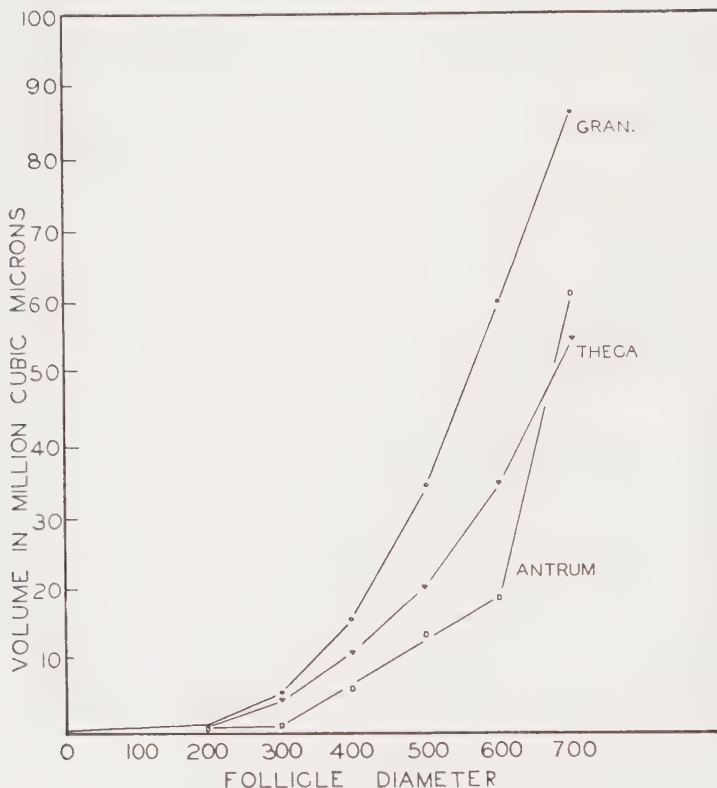


Fig.1 The volume relationships between granulosa, theca and antrum in the growth of the follicle. Each plotted point is the average for all stages of the cycle.

Mitotic activity of the granulosa (table 2, fig. 2) shows definite, cyclic, significant changes. In follicles of less than 200 μ diameter, in any stage of the cycle, the mitotic activity is at a comparatively low level. The differences which exist in the mitotic index of this size group are, with one exception,

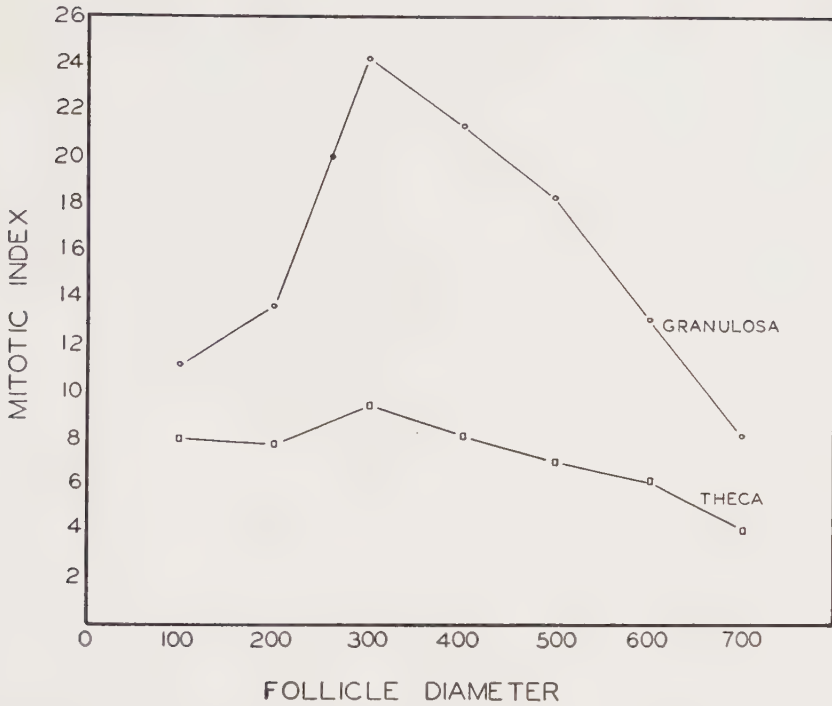


Fig.2 Mitotic activity of the granulosa and the theca during the growth of the follicle. Mitotic index is the number of mitoses to be found in 1,000,000 cu. μ of tissue.

TABLE 2

The mitotic index in the granulosa of follicles of various sizes throughout the estrous cycle

FOLLICLE DIAMETER	DIESTRUS			PROESTRUS			ESTRUS			METESTRUS		
	N	MI	PEm	N	MI	PEm	N	MI	PEm	N	MI	PEm
35-100	80	9.12	1.3	80	11.65	1.3	70	13.57	0.4	73	12.20	0.6
101-200	38	14.67	0.9	44	15.35	1.0	48	12.26	0.6	37	13.17	0.6
201-300	9	19.28	1.7	13	21.0	0.9	20	21.91	1.2	12	24.70	1.5
301-400	8	15.97	1.5	4	14.4	2.6	7	21.0	2.6	9	35.73	3.8
401-500	4	18.12	3.2	3	6.7	0.9	2	20.5	4.3	9	28.25	2.0
501-600	4	14.52	1.9	2	7.4	2.8	2	17.0	1.2	0		
601-700	3	10.40	1.1	9	5.6	0.7	5	9.36	0.7	0		
Average		14.58			11.73			16.51			22.79	

N, number of follicles studied; MI, mitotic index (mean); PEm, probable error of the mean.

insignificant. This exception occurs in diestrus when the difference between the mitotic index of follicles less than 100 μ in diameter is just significantly lower than the mitotic index of follicles between 101 and 200 μ diameter. In follicles between 200 and 300 μ diameter, in all stages of the cycle, mitotic activity is augmented significantly, and remains at a high level until the follicle has attained an over-all diameter of 400 μ , when mitotic activity undergoes a decline which is progressive until the time of ovulation is reached. The most active granulosa, judging only from mitotic activity, is to be found in ovaries of animals in metestrus, in follicles between 300 and 400 μ diameter.

TABLE 3

The mitotic index in the theca of follicles of various sizes throughout the oestrous cycle

FOLLICLE DIAMETER	DIESTRUS			PROESTRUS			ESTRUS			METESTRUS		
	N	MI	PEm	N	MI	PEm	N	MI	PEm	N	MI	PEm
35-100	16	5.7	0.21	15	8.29	0.24	15	7.97	0.32	11	10.4	0.52
101-200	20	8.1	0.31	19	7.36	0.14	16	7.87	0.30	21	7.80	0.17
201-300	8	11.36	0.36	8	9.06	0.30	12	6.88	0.22	7	10.86	0.22
301-400	8	11.2	0.37	4	5.26	0.17	8	5.82	0.31	7	10.21	0.31
401-500	6	7.4	0.26	3	5.33	0.38	0			9	8.49	0.16
501-600	4	8.1	0.17	4	4.5	0.33	0			0		
601-700	3	5.3	0.02	6	3.5	0.17	6	3.27	0.11	0		
Average		8.17			6.19			6.36			9.50	

N, number of follicles studied; MI, mitotic index (mean); PEm, probable error of the mean.

In table 3 and figure 2 is shown the mitotic activity of the theca. It is obvious that the theca is much less active in mitotic division than the granulosa and that its maxima are attained in follicles of the same size as show peaks in granulosa mitotic index. These points of maximal thecal activity are always less, quantitatively, than those exhibited by the granulosa.

Knowing the total numbers of follicles to be found in each size range, and having calculated the volume of antrum, granulosa and theca in an adequate sample of follicles from

each of these size ranges, it was a comparatively simple matter to determine the total, average volume of antrum, granulosa and theca in the entire ovary. If these values be plotted as in figure 3, taking into account the temporal values of each of the stages of the estrous cycle as given by

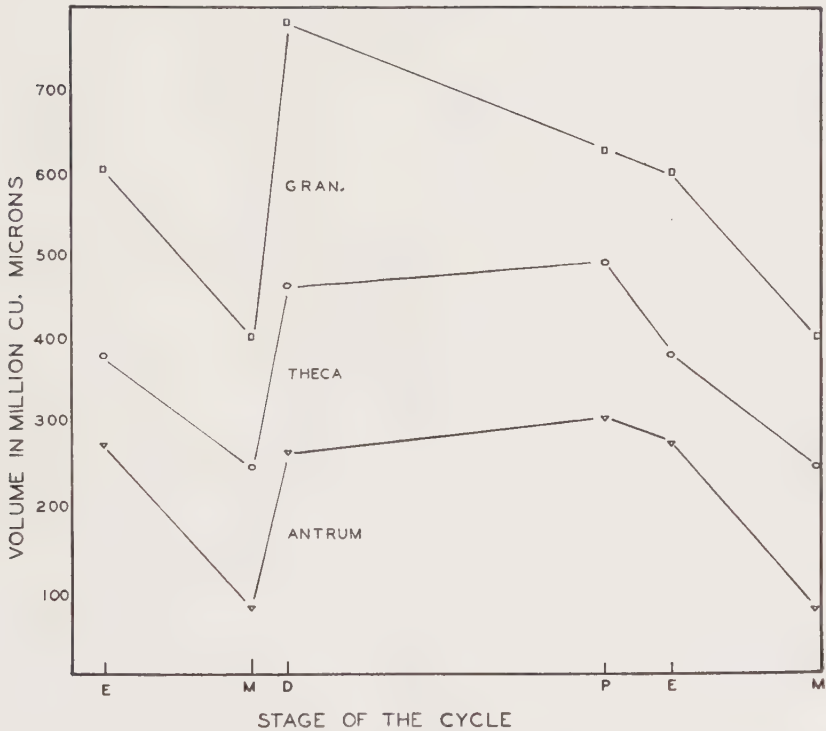


Fig. 3 Changes in total volume of granulosa, theca and antrum in the entire ovary during the estrous cycle. E is estrus, M metestrus, D diestrus and P proestrus.

Long and Evans, several interesting relationships make themselves apparent. The total volume of granulosa in the ovary almost doubles between its low point in metestrus and the diestrus peak. Thereafter follows a steady decline in volume which continues to the next metestrus period. The decline in volume is augmented during estrus. Similarly

the total volume of theca in the ovary more than doubles between its low point in metestrus and its proestrous peak. During proestrus the decrease in total theca volume is more rapid than it is after ovulation.

Changes in the total volume of antrum parallel changes in theca volume except at one point. During proestrus the total volume of antrum does not decline so rapidly as does the volume of theca.

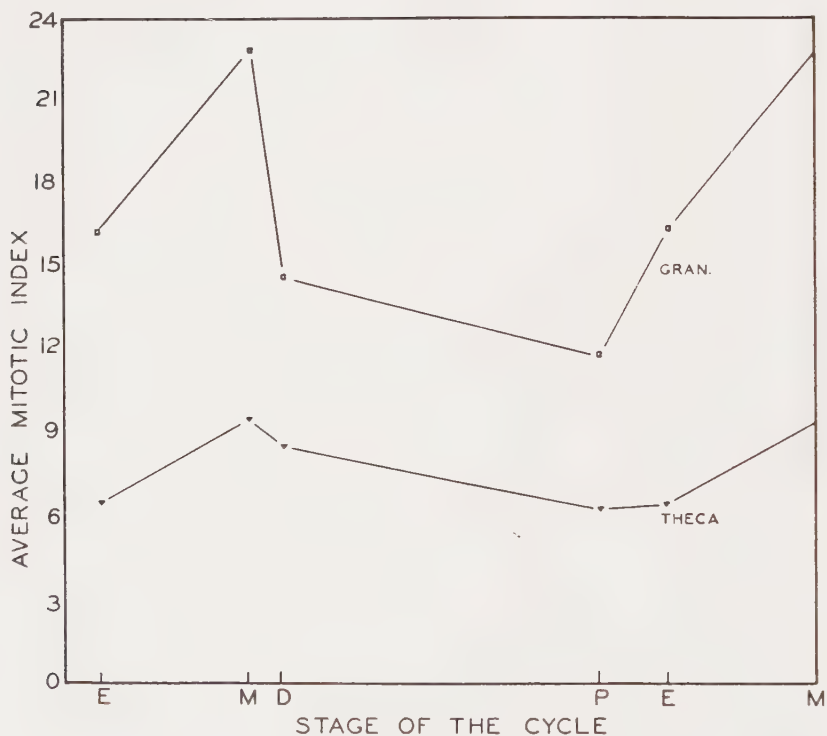


Fig. 4 Mitotic activity of the granulosa and theca during the estrous cycle. Each point represents the average for follicles of all sizes at a given stage of the cycle.

The mitotic index of the ovary as a whole, that is, the average mitotic index of the entire granulosa and thecal components of the ovary, show peaks in metestrus (fig. 4) and depressions in proestrus.

The accuracy of these averages is, admittedly, not above reproach, but they are intended to serve only as indicators for general processes.

DISCUSSION

That increase in number of small, non-antrum containing follicles in the ovary of the immature rat is due to the action of pituitary FSH was suggested by Lane ('35 a). Guthrie and Jeffers ('38) have recently reported similar increase in numbers of small follicles in the bat, after injection of 'gonadotropic hormone.' The adult rat shows, during diestrus, a dramatic increase in number of small, primary follicles. This suggests that, at this time, pituitary FSH may be liberated in increased amounts.

In the growth of the follicle (fig. 1) it is apparent that the increase in granulosa and theca volume is a regular process, going on at an almost steady rate, independent of the size of the follicle. The volume of the antrum, however, shows interesting deviations from this. When the follicle is very small, the figure for the antrum really is only the volume of the egg and its membranes. By the time the follicle reaches 300 μ in diameter, follicular fluid begins to increase in amount. From 300 to 600 μ this is a fairly steady process, whether it be actual secretion, transudation or some other process is immaterial. However, when the follicle has reached 600 μ in diameter, there then begins a very rapid increase in volume of antrum which continues until the time of ovulation. This is the 'preovulatory swelling' of various authors.

Mitotic activity is not an unfailing measure of physiologic action, but the significant changes in mitotic index shown by the granulosa are highly suggestive. The low level of mitotic activity of the granulosa of follicles less than 200 μ in diameter in all stages of the cycle, would seem to be correlated with physiologic quiescence. It has been shown (Lane, '35 a) that the ovary of the immature rat does not develop follicles larger than 200 μ until about the eighteenth day of life. Numerous investigators have demonstrated that the

ovarian response to pituitary gonadotropic stimulation before 18 days is markedly atypical, in that the ovary seems incapable of developing large follicles under most types of stimulation. Swezy ('33) and Lane and Greep ('35) showed that in the immature rat, after hypophysectomy, follicles larger than 250 μ degenerated but that follicles up to this size were maintained in an approximately normal condition. These facts might be construed to mean, for the adult, that the follicles smaller than 200 μ represent a reserve from which are drawn succeeding crops of follicles for maturation at successive estrous periods. This follicle reserve will develop or be maintained without the assistance of the pituitary, but, for the production of follicles larger than 200 to 300 μ , pituitary assistance is required.

The metestrous peak in an average mitotic index of the granulosa and theca (fig. 4) indicates the beginning of the wave of follicular growth which will culminate in the next ovulation. It seems significant that the follicles in the size range of 401 to 500 μ should exhibit the maximum mitotic activity in the granulosa. It is possible that these constitute the group which will ovulate at the succeeding estrous period. Numerically (table 1) there are six of these follicles in the average metestrous ovary. Allowing for atresia this number could easily produce the three to five ovulating follicles which are present in each ovary at estrus.

In figures 3 and 4 is seen the relationship between mitotic activity in the granulosa, its total volume, mitotic activity in the theca and its total volume, and the total volume of antrum in the ovary. Mitotic activity in both theca and granulosa reaches its peak in metestrus. The results of this activity become apparent in the production of a maximal total volume of granulosa during the next phase of the cycle, diestrus. The fact that the curve of total volume of theca continues to rise slightly after its sharp upswing in metestrus may indicate growth of the constituent cells. If the total volume of theca increases during diestrus in spite of a diminished division rate, we should have to postulate cellular

growth. The average size of the theca cells in various stages of the cycle has, however, not yet been studied.

Any discussion of the locus of estrogen formation in the rat would be, in the view of the authors, premature when based only on a descriptive study. This question should properly be postponed until the changes here reported shall have been related experimentally to pituitary function. This is now being done.

SUMMARY AND CONCLUSIONS

Using the method described herein, the total volume of granulosa, theca and antrum in the ovary of the adult rat has been determined and the changes in volume have been correlated with the estrous cycle. Volume relationships between granulosa, theca and antrum have been determined during the growth of the average follicle. Mitotic activity in the granulosa and in the theca has also been studied. The following conclusions seem warranted:

1. During diestrus the ovary is subject to increased pituitary FSH stimulation.

2. It is unlikely that the slight increase in large follicles that occurs during proestrus and estrus is sufficient to account for estrous phenomena.

3. Follicles less than 200 μ in diameter are inactive mitotically and are thought to be physiologically quiescent.

4. Between 200 and 300 μ diameter, the follicle in any stage of the cycle shows mitotic activity in the granulosa and theca which is significantly augmented. These follicles are thought to be on the way to maturation or atretic degeneration.

5. Mitotic index of the ovary as a whole is highest in metestrus.

6. Maximum total granulosa volume is seen in diestrus.

7. Maxima in the curves of total antrum volume and total volume of theca occur in proestrus.

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SUPERFICIAL LYMPHATICS OF HUMAN EYELIDS OBSERVED BY INJECTION IN VIVO

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TWO FIGURES

During studies of the linear rate of lymph flow in the superficial vessels of the eyelids, with the use of McMaster's technic of injection of patent blue V in vivo ('37), observations were made on the courses of these lymphatics.

The standard current textbooks of gross anatomy present no detailed descriptions. Even some of the special studies give little attention to the superficial lymphatics of the eyelids (Sappey, 1876; Grunert, '02; Bartels, '09; Dewey, '20; Lauber, '36). Both Whitnall ('21) and Duke-Elder ('38) describe the superficial vessels as coursing perpendicularly from the lid margins into larger trunks, which drain into the preauricular and submaxillary nodes. Only Most ('05), who injected lids of cadavers by Gerota's method, describes a horizontal alignment of the vessels. His findings are confirmed by the observations here reported.

Seventy eyelids (fifty lower and twenty upper) were studied in twenty-five normal white males, 22 to 52 years of age. The dye (0.02 cc.) was injected intradermally, at a point from 5 to 10 mm. beyond the middle of the lid margin. The wheal raised by the dye (fig. 1) precluded the demonstration of vessels at the site of injection, in an area about 3 to 4 mm. in diameter. But extending from this region the gradually filling vessels became clearly evident; they have been observed for periods as long as 75 minutes, tracings being made at 5, 10, 20 and 30 minutes following injection. Records were

made in the form of direct tracings on sheets of cellulose acetate as described by McMaster.

Many very fine dichotomously branching vessels radiate for a short distance from the margin of the injection site (fig. 2). They are presumably tributaries of the larger vessels to be described. There is considerable directional variation in the drainage of the dye into the main superficial lymphatic vessels. Filling is accomplished sometimes more fully in vessels

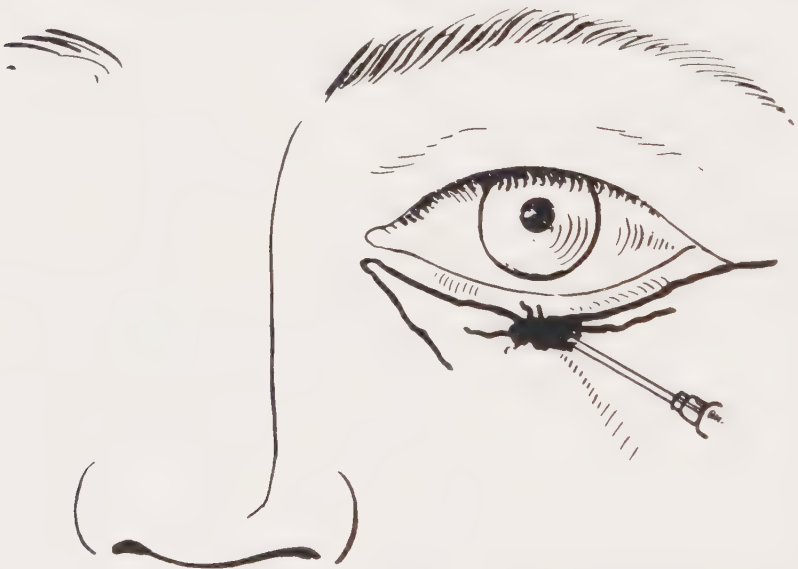


Fig. 1 An actual tracing from one subject, illustrating the wheal at site of injection and the advance of dye from this area into lymphatic vessels.

directed medially from the injection site and again, with nearly equal frequency, in vessels extending laterally from it. In the light of this variation it is evident that no claim can be made that all the major superficial vessels are demonstrated even in the combined observations. Yet it is important to note that these major vessels are found invariably to course parallel to the lid margin, in agreement with Most's findings, and not perpendicular to the margin as described by others. Figure 2 is an interpretation, based on all the avail-

able material, of what is regarded as a representative pattern of the larger superficial vessels. It appears that in either lid there are at least four main vessels, two coursing medially and two laterally. In many instances sets of three are observed, though never in the nasal portion of the lower lid. As many as four vessels occur infrequently, and then only in the lateral portion of the upper lid.

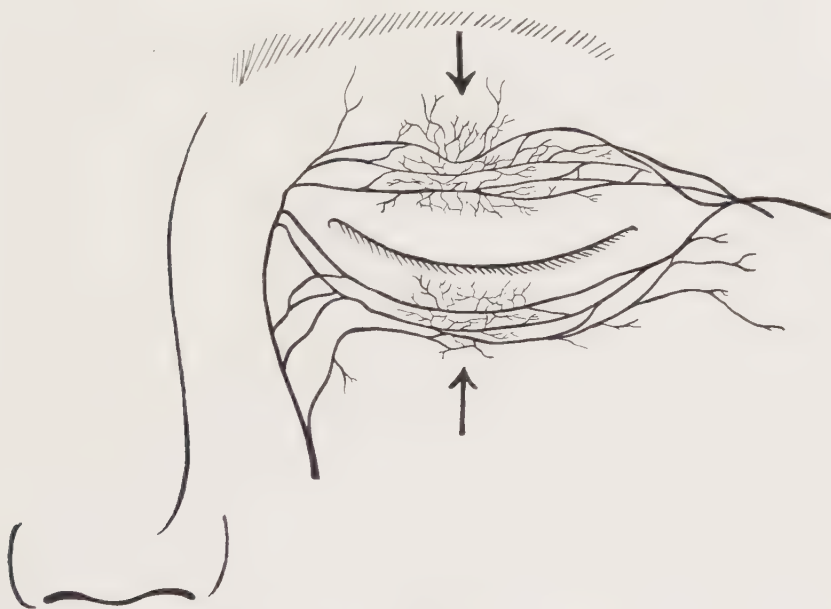


Fig.2 A composite drawing of the superficial lymphatics of the eyelids as observed in the twenty-five normal living subjects. The arrows indicate the sites of injection. The colored wheals are not indicated so that the many fine vessels which are observed at the beginning of the injections could be illustrated.

Frequently the lateral lymphatics of the upper and lower lids unite just external to the outer canthus, forming a trunk which is directed toward the preauricular nodes. The medial lymphatics of the upper and lower lids are directed medially toward a point on the nasal side of the palpebral commissure. In one instance they are seen actually to unite there, and it seems probable that this junction is characteristic, though

probably occurring at a level too deep to be ordinarily observed by the technic employed. The medial vessels of the lower lid usually can be observed to pass in close relation to the medial palpebral commissure; from a point just medial and slightly inferior to it they turn acutely, to course downward, lateral to the nose and in line with the course of the anterior facial vein as if to be continued to the submaxillary nodes.

The vessels of the upper lid are more tortuous than those of the lower. The superficial lymphatics here observed are fewer in number than is reported in description of the deeper tier of vessels lying anterior to the tarsal plate.

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THE EFFECT OF TESTOSTERONE PROPIONATE ON GONADAL DEVELOPMENT AND GONADOTROPIC HORMONE SECRETION IN YOUNG MALE RATS ¹

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ONE PLATE (NINE FIGURES)

INTRODUCTION

Following the injection of large amounts of androsterone into normal immature male rats, it has been shown that the weight of the testes is markedly decreased, the gonadotropic potency of the anterior pituitary gland is lessened, and the seminiferous tubules of the testes are damaged (Moore and Price, '37). However, in normal adult male rats, treatment with testosterone propionate produced only a slight decrease in testis weight with no histological abnormalities in the seminiferous tubules (Korenchevsky, et al., '37). It has been reported also that estrone, dehydroandrosterone, testosterone and testosterone propionate will prevent the increase in gonadotropic potency of the pituitary gland of immature castrated male or female rats united in parabiosis with a normal female partner (Meyer and Hertz, '37; Hertz and Meyer, '37). In view of the effects produced by the administration of these male and female hormones, it became of interest to determine the effect of the injection of testosterone propionate ² in varying dosages and for different periods of time upon the gonads and pituitary gland of newborn and immature male rats.

¹Supported in part by a grant from the Wisconsin Alumni Research Foundation administered by Roland K. Meyer.

²We are indebted to Dr. Erwin Schwenk of the Schering Corp. for the generous supply of testosterone propionate.

PARABIOSIS STUDIES

Meyer and Hertz ('37) have demonstrated the suitability of the parabiosis method for quantitatively studying gonadotropic secretion following castration. Accordingly, the method described by them was used in a part of the present studies. Eleven male rats were injected once daily with 2 γ , and a like number with 10 γ of crystalline testosterone propionate, in 0.01 cc. of corn oil from the day of birth until 31 days of age. The injections were then stopped, the animals were castrated and united in parabiosis with a normal female

TABLE 1

The effect of testosterone propionate on the secretion of gonadotropic hormone in parabiotic rats

NUMBER OF PAIRS	DAILY DOSE γ	NUMBER OF DAYS IN PARABIOSIS	LEFT PARTNER		RIGHT PARTNER	
			Mean ovarian weight	Pituitary weight	Mean testes weight ¹	Pituitary weight
			<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
11	10	13-36 ²	16 ³	4.5	71	4.7
9	2	10	121	5.6	512	5.0
11	None	10	117	4.4	784	4.7

¹ Testes weights are weights at the time of castration which is 31 days of age in all cases.

² The average time of parabiotic union was 23 days.

³ The weight of the ovaries of the pair which showed some stimulation was 64 mg. and follicles and corpora lutea were present.

littermate. The uninjected control pairs and those injected with 2 γ of testosterone propionate were autopsied after remaining in parabiosis for 10 days. The pairs injected with 10 γ of testosterone propionate remained in parabiotic union for 13 to 36 days.

Table 1 shows that 2 γ of crystalline hormone per day administered from birth until 31 days of age, did not prevent the characteristic hypersecretion of gonadotropic hormone by the pituitary gland that occurs following 10 days of castration. On the other hand, 10 γ per day did prevent the increase in the quantity of gonadotropic hormone. At the end of 10 days

in parabiosis, the vaginas of the female partners of the latter group were not open. It was then decided to continue these pairs until the vaginas did open. In only one case did this occur, and that after being in parabiosis for 31 days. This pair was the only one of this group that showed any stimulation of the ovaries of the female partner. The uninjected control pairs gave an average ovarian weight very near that of the pairs treated with 2 γ of testosterone propionate. There was no significant difference in the weight of the pituitary glands of each of the three groups. There was, however, a very decided difference in the testis weights of the rats treated with 10 γ of testosterone propionate and the controls. Apparently 10 γ of hormone was sufficient to prevent marked testis growth up to 31 days of age (the time at which these animals were castrated and put in parabiosis). The increase in testis size from that at the day of birth (4.7 mg., average of twenty-six rats) to that at 31 days of age in the rats receiving 10 γ of hormone (71 mg.), is approximately one-eleventh the weight increase of the testes of normal rats during the same period of time. Histological examination showed that the seminiferous tubules of the injected rats were smaller and closer together than those of normal animals. Furthermore, in the tubules of the injected animals, mitoses were not as numerous as in controls, and there appeared to be a sloughing off of the cellular elements into the lumen of the tubules (figs. 1, 2).

INJECTIONS OF TESTOSTERONE PROPIONATE FOR VARYING PERIODS OF TIME

In the rats used in the parabiosis studies, it was observed that the testes of animals injected with 2 γ of male hormone per day descended on the average of 7 days earlier than did the testes of normal rats (table 5). This observation suggested the possibility of inducing precocious spermatogenesis after continued treatment with 2 γ of hormone per day. Accordingly, twelve male rats were injected with 2 γ of testos-

terone propionate per day from the day of birth until 36, 40 and 44 days of age.

Table 2 shows that although 2 γ of hormone was sufficient to partially inhibit the growth of the testes, it had no effect in accelerating or delaying sperm development since mature sperm were present in the tubules of these rats at 44 days of age which is the time they appear in the testes of normal males of our colony. Histological study of the testes revealed no noticeable effects of the hormone upon the germinal epithelium (figs. 3, 4). The accessory glands of the treated rats of this series were smaller than those of the controls. Since the size of the accessories is an index of the amount of male

TABLE 2
Testes and accessory weights of male rats treated from birth with 2 γ of testosterone propionate

NUMBER OF ANIMALS	NUMBER OF DAYS INJECTED	TESTES WEIGHT	MATURE SPERM IN TESTES	SEM. VESC. WEIGHT	PROSTATE WEIGHT
		<i>mg.</i>		<i>mg.</i>	<i>mg.</i>
4 (treated)	36	670	No	23	59
4 (controls)	36	1133	No	30	77
3 (treated)	40	880	No	23	75
4 (controls)	40	1419	No	34	72
5 (treated)	44	1270	Yes	42	104
3 (controls)	44	1730	Yes	59	111

hormone acting upon them (Moore, Hughes and Gallagher, '30), it appears from these data that the injected male hormone plus that secreted by the animal's own gonads was not enough to maintain the accessories in a normal condition. When 10 γ per day was used, this was likewise the case in all the animals excepting those that were injected for 31 days (table 4).

The injury to the seminiferous tubules of the rats treated with 10 γ of testosterone propionate per day led us to determine whether the testes of such animals would respond to injections of pituitary extracts, and whether the animal's own pituitary gland would stimulate the testes after injec-

tions of male hormone were discontinued. Fifteen male rats were injected with 10 γ of testosterone propionate per day from the day of birth until 31 days of age. Injections of an unfractionated aqueous extract of sheep pituitary glands were then begun and continued for 15 days in one group of animals and for 30 days in a second group. Animals which had received only the male hormone for 31 days were autopsied with each group. Rats which received the testosterone propionate for 31 days plus the pituitary extract for 15 days, and rats which received only the testosterone propionate for 31 days, showed no evidence of sperm development when autopsied at 46 days of life. Rats which received the male hormone for 31 days plus the pituitary extract for 30 days likewise did not have any mature sperm in the seminiferous tubules at the time of autopsy. There was, however, a great amount of interstitial tissue development and stimulation of the accessory glands in these rats (figs. 5, 6). In the tubules of two of the rats which received only the male hormone for 31 days, there were occasional sperm heads at 61 days of age. The testes of this entire series of animals were definitely damaged for the tubules were small and there was a sloughing of the cellular elements into the lumen of the tubules. The gonads of the rats which received only the male hormone for 31 days had increased in size from 71 mg. (weight at 31 days) to 192 mg. at 46 days, and to 462 mg. at 61 days. This weight increase indicates that the animal's own pituitary gland had begun to produce gonadotropic hormone, especially between 46 and 61 days of age. Male hormone production, however, was at a subnormal level at these ages as is indicated by the accessory organ weights (table 3).

In order to determine the effect of prolonged injection of 10 γ of testosterone propionate per day, three groups of rats were treated from birth for 31, 51 and 81 days. Table 4 shows the results obtained. The testes of the treated rats weighed much less than did those of the controls at all ages. There was a very decided difference in the numbers of mature sperm produced in the animals treated for 51 days and in those

TABLE 3

Testes and accessory weights of male rats treated with testosterone propionate and testosterone propionate plus gonadotropic extract

NUMBER OF ANIMALS	TREATMENT	AGE AT AUTOPSY	TESTES WEIGHT	SEM. VESC. WEIGHT	PROSTATE WEIGHT
		<i>days</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
2	10 γ Tp per day from birth until 31 days	46	192	22	26
2	10 γ Tp per day from birth until 31 days plus 100 mg. SAP per day from 31 to 46 days	46	653	44	126
5	10 γ Tp per day from birth until 31 days	61	462	18	27
6	10 γ Tp per day from birth until 31 days plus 100 mg. SAP per day from 31 to 61 days	61	835	104	383

TABLE 4

Testes and accessory weights of male rats injected from birth with 10 γ of testosterone propionate for varying periods of time

NUMBER OF ANIMALS	TREATMENT	AGE AT AUTOPSY	TESTES WEIGHT	MATURE SPERM IN TESTES	SEM. VESC. WEIGHT	PROSTATE WEIGHT
		<i>days</i>	<i>mg.</i>		<i>mg.</i>	<i>mg.</i>
12	10 γ Tp per day from birth until 31 days	31	71	No	27	73
11	none (controls)	31	784	No	16	68
2	10 γ Tp per day from birth until 51 days	77	1184	Some sperm heads	75	106
2	none (controls)	77	3140	Yes	459	315
4	10 γ Tp per day from birth until 51 days	164	1730	Yes	108	102
3	none (controls)	164	3399	Yes	1076	870
2	10 γ Tp per day from birth until 81 days	81	1349	Yes	119	139
2	none (controls)	81	3045	Yes	693	466
3	10 γ Tp per day from birth until 81 days	152	1977	Yes	175	221
3	none (controls)	152	3215	Yes	1039	774

treated for 81 days when they were autopsied at 77 and 81 days of age respectively. In the former there were only a few sperm in the seminiferous tubules, while in the latter the numbers of mature sperm appeared to approximate the numbers in untreated controls. The seminal vesicle and prostate weights of the treated rats were less than those of the controls at all ages except at 31 days of age. The interstitial tissue of the normal rats at this age was producing less than the equivalent of 10 γ of testosterone propionate per day, as is shown by the smaller weights of the seminal vesicles of these rats.

Histological examination showed that the epithelium of the accessories of the treated rats was not as high as the epithelium of the accessories of control rats at 77 and 81 days of age (figs. 7, 8). The difference in the accessory weights of these two groups of rats was no doubt largely due to the lack of secretion in the accessories of the treated animals, for there was usually little if any secretion present at autopsy.

Histological study of the pituitary glands of all the treated rats of this series showed some degranulation of the basophils of the anterior lobe. Wolfe and Hamilton ('37) have previously reported similar effects upon the pituitaries of young male and female rats injected with testosterone propionate.

EFFECT OF TESTOSTERONE PROPIONATE UPON THE TIME OF TESTES DESCENT

The time at which the testes of all rats used in these experiments descended into the scrotum was recorded after daily observation. Table 5 shows that descent was hastened with 2 γ of testosterone propionate per day and delayed with 10 γ per day. From the reports in the literature (Donaldson, '24; Einhorn, '38 and Einhorn and Rowntree, '38) testes descent occurs between 30 to 40 days of age in normal male rats of other colonies. The animals of our colony are apparently very precocious in this regard, for the testes usually descend into the scrotum around 15 days of age. The scrota of the group of rats injected with 10 γ of hormone did not show normal hyperemia and turgescence. For the

purpose of obtaining an approximation of the relative amount of scrotal development, the distance from the anus to the point where the hair was first present along the midline of the scrotal area was measured. It was found that there was no measurable difference between these points in the animals treated with 2 γ of male hormone and the controls. This distance, however, in the animals receiving 10 γ was less than in the other two groups, and the testes were smaller. This would seem to indicate that the size of the testes is, of itself, at least a contributing factor in scrotal development. Cutuly and Cutuly ('38) have suggested that the degree of turgescence and hyperemia of the scrotum of a hypophysectomized parabiont is a sensitive indicator of endogenous male

TABLE 5
Time of testes descent of rats injected with testosterone propionate from the day of birth

NUMBER OF ANIMALS	DAILY DOSE	AVERAGE TIME OF DESCENT	RANGE IN DAYS
	γ	<i>days</i>	
22	2	8.2	7-13
18	10	24.6	15-38
26	None	15.1	13-19

hormone production following the castration of the other partner. Our data show that when a continued small dosage of testosterone propionate was injected into newborn rats, the scrotal area developed normally and could not be distinguished from that of normal animals. When larger doses were used, however, the amount of scrotal development was less than in normal animals. These data may indicate that some androgen other than testosterone propionate is responsible for the development of the scrotum in intact animals, for this area was always underdeveloped when the larger doses of testosterone propionate were used.

In another series of animals the testes were allowed to descend normally (which was 17 to 18 days in these particular rats) and then male hormone injections were begun (table 6).

With a daily dose of 10 γ and 50 γ of testosterone propionate, the testes remained in the scrotum throughout the injection period, which was 30 days, and the testis weights of rats receiving 50 γ were much less than those of animals receiving 10 γ . Conversely, the accessory weights were increased more with 50 γ than they were with 10 γ of the male hormone. The accessories of the normal animals, however, weighed more than did those of animals treated with 10 γ of hormone. The amount of scrotal development again seemed to be correlated with the size of the testes, for there was much more hair present over the scrotal area of the animals with small testes (those receiving 50 γ) than over the scrotal area of those with larger testes (those receiving 10 γ). The latter group

TABLE 6

Testes and accessory weights of male rats treated with testosterone propionate after the testes had descended

NUMBER OF ANIMALS	DAILY DOSE	AGE AT AUTOPSY	TESTES WEIGHT	SEM. VESC. WEIGHT	PROSTATE WEIGHT
	γ	days	mg.	mg.	mg.
5	10	47	1315	77	131
6	50	47-48	326	266	264
7	None	45-48	2042	101	170

did not show the degree of scrotal development present in the controls.

Since the time of testis descent was delayed by the administration of 10 γ of testosterone propionate from the day of birth, it was thought possible to delay descent for a longer time if larger dosages of male hormone were used. Accordingly eighteen male rats from three litters were treated from birth with 10 γ of hormone per day until 24 days of age (table 7). At this time the testes of a few of the rats showed evidence of beginning descent. The dosage was raised to 20 γ per day which prevented further descent until 49 days of age. Here again there was evidence in a few of the rats that their testes were beginning to descend so the dosage was raised to 35 γ per day and continued for 12 days. At 61 days of

age the daily dosage was raised to 50 γ and continued until 77 days of age. At 70 to 75 days of age the testes of all the animals were completely descended despite the hormone injections. The last 5 days of treatment the animals received 100 γ of hormone per day, but the testes remained in the scrotum once they had descended. The seminiferous tubules of twelve of these rats did not have any sperm (fig. 9); those of five had a few sperm; and those of one rat had numerous sperm at the time of autopsy.

TABLE 7

Testes and accessory weights of male rats treated from birth with increasing amounts of testosterone propionate

NUMBER OF ANIMALS	AGE AT AUTOPSY	TESTES WEIGHT	SEM. VESC. WEIGHT	PROSTATE WEIGHT
	<i>days</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
18 (treated)	80-84	555	315	278
8 (controls)	77-81	3093	576	390

DISCUSSION

It has recently been reported (Diaz, et al., '38), that the pituitary glands of adult female rats injected with estrin for long periods of time do not stimulate the ovaries of immature mice when implanted into the latter. It was concluded that the estrin caused exhaustion of the pituitary gland and for that reason there was not sufficient gonadotropic hormone contained in the glands to give an ovarian response. The results of the parabiotic studies reported herein show that the pituitary glands of male rats treated from birth with sufficient male hormone did not undergo castration hypersecretion for at least 36 days after the treated rats were castrated and the injections of testosterone propionate stopped. These data may be interpreted as supporting the theory of Diaz et al. Apparently the pituitary gland of immature rats recovers very slowly the ability to secrete gonadotropic hormone after the suppressing effect of the testosterone propionate is removed. It also seems probable that there never was a complete recovery from the effects of the male hormone

even after the injections were discontinued, for the testes and accessories of animals injected with testosterone propionate were always smaller than those of untreated rats at every age at which the animals were autopsied.

The results obtained from the study of single rats treated for long periods of time with a constant dosage of male hormone suggests that the suppression of gonadotropic secretion was not complete, especially toward the end of the injection period. The evidence for this is that at 81 days of age there was an abundance of sperm in the tubules, while at 61 days of age usually only a very few sperm heads were present. There was evidently a gradual increase with age in the amount of gonadotropic hormone secreted when the dosage of male hormone remained constant. If the dosage was increased as the animals grew older, gonadotropic secretion was further suppressed so that mature sperm did not appear in the seminiferous tubules until 80 to 84 days of life and the testes did not descend into the scrotum until 70 to 75 days of life.

If male hormone alone is the agent responsible for descent of the testes, as has been reported (Hamilton, '37), it is difficult to understand why the administration of the large doses of testosterone propionate in our experiments prevented descent rather than facilitated it, for the greater the amount of male hormone injected, the longer was testes descent prevented. We have determined in untreated rats hypophysectomized at 31 days of age, that 40 γ of testosterone propionate per day is required to bring about descent when the injections are begun on the forty-first day of life (unpublished data). The dosage necessary to cause testis descent in untreated hypophysectomized rats is thus less than the dosage necessary to prevent descent until 70 days of age in the rats treated with increasing amounts of male hormone.

A possible explanation for the early descent of the testes following treatment with 2 γ of hormone per day is that this quantity was not enough to suppress the pituitary gland, but

when added to the male hormone being produced by the testes, it was sufficient to facilitate descent.

Moore and Price ('37) have previously reported harmful effects upon the seminiferous tubules of immature rats treated with large doses of androsterone. They consider the injury an indirect effect due to a decrease in gonad-stimulating hormone of the pituitary gland, for the pituitaries of treated animals showed a decreased potency when implanted. In our studies the pituitary glands of animals treated with relatively small doses of testosterone propionate per day did not undergo castration hypersecretion, testis growth was greatly inhibited, harmful effects upon the seminiferous tubules were observed, and the time of appearance of sperm was delayed. These effects are no doubt the result of the production of subnormal amounts of gonadotropic hormone by the pituitary gland of the treated rats. Moreover, this is indicated further by a quantitative consideration of the amount of growth of the testes of treated rats as compared with controls. The ratio of the testis weight of animals treated with 10 γ of hormone per day from birth until 31 days to the testis weight of normal rats was 1:11. After the testes had descended normally into the scrotum (18 days) and the animals were treated with 10 γ of hormone per day for 30 days, this ratio was 1:1.4. If 50 γ per day was given during the same period, the ratio was 1:6.2. When the 10 γ dosage was injected from birth until 81 days, the ratio of testis weight of injected animals to testis weight of controls was 1:2.2. These figures indicate that the degree to which testis growth is prevented by male hormone treatment is dependent upon the age of the animals and upon the dosage of male hormone used. Further support for this idea can be found in the report of Korenchevsky who found that large doses of testosterone propionate (1500 γ) had little effect on the testes of adult male rats.

It has been demonstrated that the amount of growth of the accessory glands is dependent upon the quantity of male hormone acting upon them (Moore, et al., '30; Korenchevsky,

et al., '36), and that the quantity of male hormone produced is dependent upon the amount of gonadotropic hormone (presumably luteinizing principle) acting upon the interstitial tissue of the testes (Greep and Fevold, '36; Greep, Fevold and Hisaw, '37). From our results with animals treated for long periods of time, it appears that the injected male hormone plus that secreted by the animal's own gonads was not sufficient to maintain normal accessories (table 4). When the injections were stopped and the animals allowed to live, the accessories and testes of these rats never did attain the same weight as the accessories and testes of normal animals. This might be explained in one of three ways: 1) that the accessory gland epithelium was rendered refractory to stimulation by male hormone; 2) that the interstitial tissue of the gonads was refractory to stimulation; or 3) that the pituitary gland was rendered incapable of producing sufficient gonadotropic hormone to stimulate the interstitial tissue. The latter seems the most likely explanation, for the results reported in table 3 indicate that the accessories and the interstitial tissue were not refractory to stimulation, for both were stimulated following the injection of unfractionated pituitary extracts.

The exact mechanism of action of the testosterone propionate upon the testes and accessory glands of rats in these experiments is not known. It is indicated, however, that the testosterone propionate was administered in dosages optimal for preventing all, or nearly all, gonadotropic secretion from the pituitary gland, especially at the younger ages. As a result, the animal's own gonads did not contribute any male hormone, or at best, very little. The injected hormone, consequently, was insufficient to maintain normal accessories, but was sufficient to suppress the pituitary gland. After the injections were stopped, the data indicate that some gonadotropic secretion was present for the accessories enlarged. The male hormone produced as a result of this gonadotropic secretion no doubt acted upon the pituitary and inhibited excessive gonadotropic secretion, and thus prevented the

testes and accessory glands from compensating for the growth they had lost during the time they received relatively little stimulation. Further studies are needed, however, to clarify these relationships.

SUMMARY

Using the parabiosis technique it was determined that the pituitary glands of male rats injected from birth with 10 γ of testosterone propionate per day did not show castration hypersecretion following gonad removal, while the pituitaries of normal rats and those injected with 2 γ did undergo hypersecretion.

Normal growth and development of the testes of the rats was prevented by injection of 2 to 50 γ of testosterone propionate from the day of birth. The larger doses injured the seminiferous tubules and delayed the time of appearance of mature sperm, while the smallest dose partially inhibited testis growth. After the injections were discontinued, the testes and accessory glands of the rats which had received testosterone propionate from the day of birth did not attain the same weight as the corresponding glands of normal animals.

Rats that received 2 to 50 γ of testosterone propionate per day from birth had smaller accessory glands than the controls at every autopsy age with the exception of those that were injected for 31 days with 10 γ per day. The dosage of testosterone propionate required to maintain normal accessories was greater than that needed to prevent gonadotropic secretion. The accessories and interstitial tissue of animals injected with testosterone propionate from birth were greatly stimulated following subsequent injection with pituitary extract.

Testis descent was hastened with daily doses of 2 γ and prevented with 10 to 50 γ of testosterone propionate for as long as 55 to 60 days after normal descent occurred. The scrotal area of rats receiving the larger doses was not as well developed as that of normal rats.

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PLATE 1

EXPLANATION OF FIGURES

1 Section of a testis of a rat injected from the day of birth until 31 days of age with 10 γ of testosterone propionate per day. $\times 300$. Note the crowding of the tubules and their atypical appearance.

2 Section of a testis of an uninjected control rat at 31 days of age. $\times 300$.

3 Section of a testis of a rat injected from the day of birth until 44 days of age with 2 γ of testosterone propionate per day. $\times 300$.

4 Section of a testis of an uninjected control rat 44 days of age. $\times 300$.

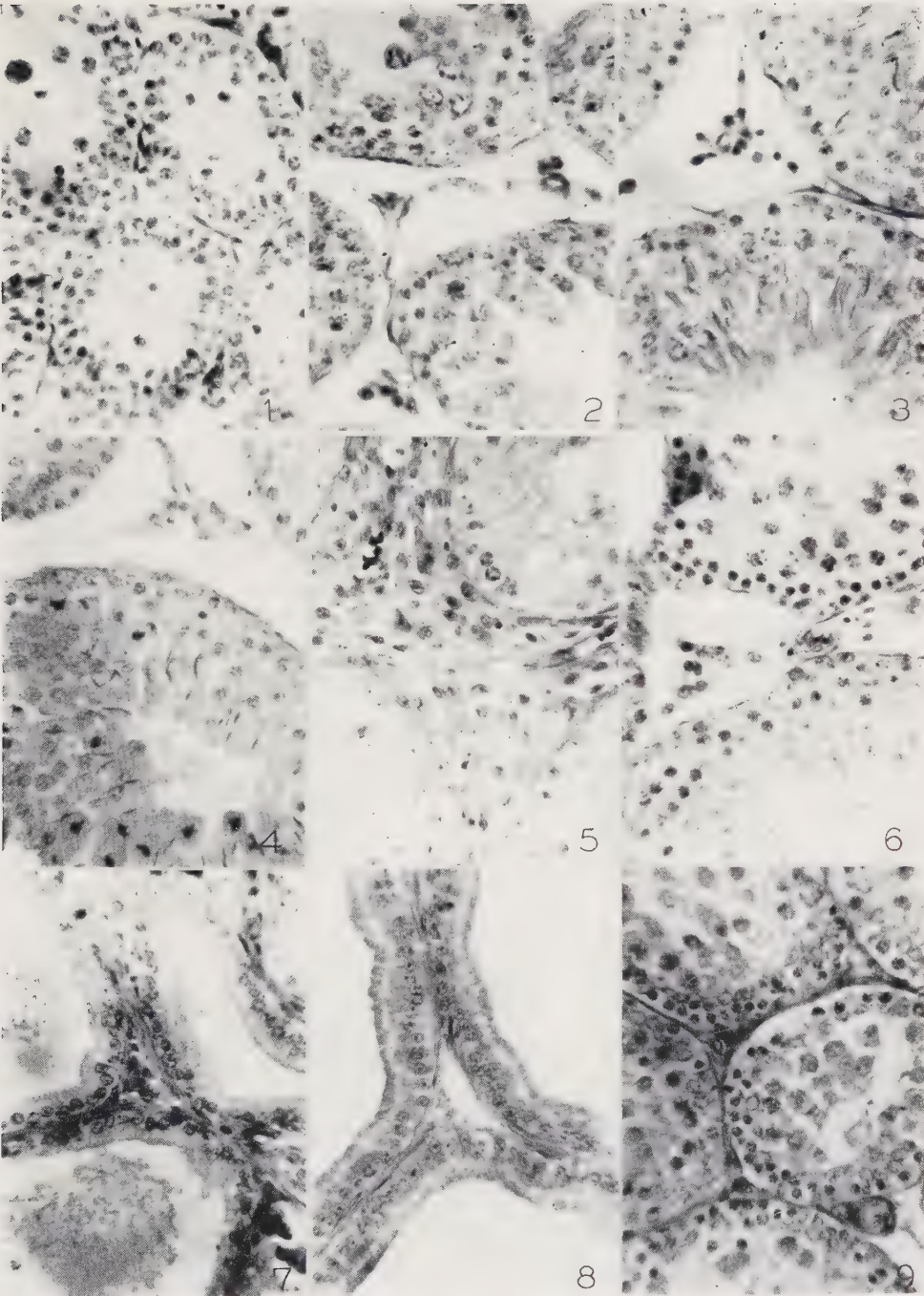
5 Section of a testis of a rat injected from the day of birth until 31 days of age with 10 γ of testosterone propionate per day followed by injections of an unfractionated aqueous extract of sheep pituitary glands from 31 to 61 days of age. $\times 300$. Note the increased interstitial tissue and tubular injury.

6 Section of a testis of a rat injected from the day of birth until 31 days of age with 10 γ of testosterone propionate per day and autopsied at 61 days of age. $\times 300$. Note the amount of interstitial tissue as compared with the previous figure and the tubular injury.

7 Section of the prostate gland of a rat injected from the day of birth until 51 days of age with 10 γ of testosterone propionate per day and autopsied at 77 days of age. $\times 300$. Note the absence of the Golgi apparatus in the epithelial cells.

8 Section of the prostate gland of an uninjected control rat 77 days of age. $\times 300$. Note the position of the Golgi apparatus of the epithelial cells near the lumen of the acinus.

9 Section of a testis of a rat injected from the day of birth until 84 days of age with increasing doses (10 to 100 γ per day) of testosterone propionate. $\times 300$. Note the crowding of the tubules and the sloughing off of the cellular elements into the lumen.



A NEW SILVER METHOD FOR STAINING NERVE FIBERS IN BLOCKS OF NERVOUS TISSUE

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ONE PLATE (TWO FIGURES)

INTRODUCTION

In 1936 the author reported before the American Association of Anatomists a new silver method for staining axons in blocks of nervous tissue. At that time it was demonstrated that the composition of the fixing fluid was important if clear-cut separation and critical staining of the nerve fibers, particularly the unmyelinated axons, was to be secured. It was shown that not only could axons be stained with silver after fixation in aqueous solutions of chromium salts, suitably modified by the addition of basic compounds, but it was also emphasized that the type of base was significant. Better fixation was secured if the chromate solutions were modified with pyridine than with ammonia, and still better results seemed to be obtained if both pyridine and ammonia were added to the chromate solutions. The addition of methyl or ethyl alcohol to the formula also appeared to be beneficial. Recently Bodian ('37) confirmed the above findings by demonstrating that sections of nervous tissue, fixed with solutions containing potassium bichromate, could be successfully silvered on the slide with protargol if the fixing fluid was 'rectified' with ammonium hydroxide and pyridine.

Since the earlier report, efforts have been directed not only toward the refinement of the method for peripheral nerves, for which it was originally devised, but also toward its application to blocks of tissue from the central nervous system.

These later researches have resulted in the development of the present method which adequately segregates and stains the smallest fibers of both the central nervous system (fig. 2) and the cranio-spinal nerves (fig. 1).

The technique has been designed to meet the requirements of cytological procedures and therefore is of value in the quantitative analysis of the axons in peripheral nerves because of its selective staining and segregation of the smallest fibers. Likewise, since it stains the fibers in both the central and peripheral nervous system in a manner similar to that effected with the Ranson pyridine silver technique (large fibers light, small axons dark) it is believed that the method will be a valuable companion technique to the Ranson method wherever the latter is indicated.

TECHNIQUE FOR PERIPHERAL NERVES

Nerves are prepared for fixation by the method previously described by the writer (Foley, '38). After the nerves have been suitably stretched they are fixed in the ice box at 4°C. for 24 hours in the following mixture:

	cc.
Potassium bichromate (3 to 5% aqueous solution)	49
Ammonium hydroxide (28%)	1
Pyridine (concentrated)	15
Ethyl alcohol (95%)	50

At the end of the period of fixation the tissue, on the stretchers, is washed for 15 to 30 minutes in the following grades of alcohol (50, 40, 30, 20 and 10%); to each of which 15 cc. of concentrated pyridine per 85 cc. of alcohol has been added. Excess bichromate is extracted by this procedure so that, by the time the tissue reaches 10% pyridinated alcohol little or no yellow should appear in the extractive. Therefore, the amount of time, whether 15 or 30 minutes, in which the nerves should remain in each grade of pyridinated alcohol can only be determined by trial. Following the washes in the pyridinated alcohols the nerves are passed through grades of aqueous pyridine to 50% pyridine by increments of 10%,

remaining in each percentage for 15 to 30 minutes. At the end of the interval in 50% pyridine the tissue is removed from the stretchers and the knot and the portion of the nerve it embraces is cut from the end of the nerve from which sections are desired. The nerve is then threaded through a piece of fixed cerebral cortex, strips of which had been preserved in 50% pyridine when the animal, from which the nerves were secured, was sacrificed. Although superior results are obtained with strips of cortex, pieces of fixed spinal cord give satisfactory results, especially if the nerve is drawn through the lateral gray column. The coated nerve is now passed through 60, 70, 80 and 90% aqueous solutions of pyridine, remaining in each percentage grade for at least 30 minutes.¹ Reasonably long intervals in pyridine are not deleterious to subsequent staining with silver. The coated nerves can remain overnight in one of the higher concentrations of pyridine without damage. After 90% pyridine the tissue is placed in absolute pyridine for 24 hours. During this 24-hour interval the pyridine should be changed two or three times to remove all the water. If this is not done the coating around the nerve is apt to split during succeeding steps.² Following the 24-hour interval in concentrated pyridine the tissue is removed and trimmed with a sharp razor to a diameter of 3 to 5 mm., after which it is passed from absolute pyridine through grades of pyridine by decrements of 10%, remaining in each percentage for 15 to 30 minutes. From 10% aqueous pyridine the coated nerve is put into distilled water where it is allowed to remain for at least 36 hours,

¹The aqueous pyridine solutions may be used repeatedly. The lower grades (up to 50%) extract the fixing fluid which was not removed by the pyridinated alcohols and should be discarded when they become decidedly yellow. The pyridinated alcohols can be used only once.

²If the coating splits during washing, it can be repaired by filling the crack with cerebellar cortex, finely chopped with scissors. The cortex is removed for this purpose and stored in saline in the ice box at the time the animal is killed. Calking is done by gently blotting off the excess water from the nerve and pressing the pasty material into the crack with a dissecting needle. It is sealed in position by allowing a drop of silver nitrate (from a medicine dropper) to flow over it before it is dropped into the vial of silver nitrate.

the water being changed every half hour during the working day. After washing, the tissue is put into a 2.0% aqueous solution of silver nitrate and kept in the oven, at 37.5°C., for 3 to 5 days. At the end of the silvering period the coated nerve is washed for a few seconds with distilled water and put into Ranson's ('12) pyro-formalin reducer for 48 hours. After reduction the tissue is dehydrated with alcohol and, if thin sections with cytological detail are desired, it is embedded according to the method recently recommended by the writer (Foley, '38). However, if thick sections (10 to 20 μ) are wanted, then some good paraffin technique (butyl alcohol) is recommended since minute wrinkles are difficult to avoid in thick sections of double-embedded material. Sections are cut and spread in the usual fashion. However, as emphasized elsewhere (Foley, loc. cit.) if the sections are to be used for fiber counts the recommended procedure (Foley, loc. cit.) is to tone and counterstain some preparations under microscopic inspection to make sure that pale fibers are not being missed. Sometimes, although every precaution is observed and the preparation is most carefully watched under the microscope, the unmyelinated fibers cannot be individualized by the toning process. The Schwann sheath material in which they are embedded is also argentophilic, and closely packed axons often appear as single or groups of presumably large unmyelinated fibers. Such heavily impregnated preparations need not be discarded. The small fibers in these clumps can be separated with clarity by destaining the preparation before toning with Kultschitsky's reagent (0.1% potassium ferricyanide in a 0.5% aqueous solution of lithium carbonate) or 20% nitric acid (Weiss, '35); the writer prefers the former. Treatment with these reagents sometimes causes the sections to slip off or wrinkle; this can be avoided by giving the sections a thin coating of celloidin (0.5%) before they are destained. If sections are bleached in this manner, they must be thoroughly washed in water before they are toned and the coating of celloidin must be removed before the fast green and orange G components of the counterstain are applied.

METHOD FOR CENTRAL NERVOUS SYSTEM

The central nervous system is perfused with fixing fluid after the blood has been washed from the vessels with normal saline. Perfusion should take place slowly, over a period of 1 to 2 hours. Precautions should be taken during the first 30 minutes of the perfusion period to determine whether the fixation is successful. Coloration of superficial structures is usually a fair indication of success. At the termination of the interval of perfusion, the parts of the central nervous system desired for study are dissected and placed in fresh fixative in the ice box for approximately 48 hours.

Following fixation the tissue is passed through the percentages of pyridinated alcohol and pyridine as previously described, remaining 1 hour in each grade. After 24 hours in concentrated pyridine, the blocks of tissue are passed through decrements of pyridine to water. They are then washed for about 16 hours (overnight) in tap water and 24 hours in many changes of distilled water. From water the tissue is put into a 2.0% aqueous solution of silver nitrate for 5 to 7 days, one change of silver being made during impregnation. After impregnation the blocks are washed very briefly in distilled water and reduced for 2 days at room temperature in Ranson's pyro-formalin mixture.

Following reduction the tissue is passed through successive grades of alcohol to absolute by increments of 10%, remaining in each percentage for a minimum of 30 minutes. From absolute alcohol the tissue is passed through either butyl alcohol, benzene, or xylol and then into paraffin. Dioxan should be avoided as a dehydrating and embedding medium as it extracts the silver from the periphery of the block. For superior results the drop method should be utilized for clearing and embedding. Double embedding should not be attempted unless the solutions of thin and thick celloidin are saturated with extractive (decided brown color) from blocks of coated peripheral nerves which have been subjected to the double embedding technique. Freshly made solutions, especially the more dilute concentrations, tend to extract the

silver from the axons at the periphery of the block. Sections are cut and spread on albuminized water in the usual manner. For routine examination the paraffin is dissolved, when the sections are dry, and the preparation is mounted in damar. If one wishes to study the non-nervous structures or use the preparations for photography then either toning alone or toning with subsequent counterstaining (Foley, '36, '38) is indicated.

DISCUSSION

The demonstration, that reduced silver preparations can be successfully destained, brings silver staining in line with routine histological and cytological staining techniques; so that, the problem of over-impregnation becomes of less consequence than that of under-impregnation and in most instances may be the desired rather than the undesired result. The following procedure is routine in the writer's laboratory and is here presented as a possible working basis for the study of nerves which contain considerable numbers of unmyelinated fibers. Several slides with sections affixed are prepared and of these, one is counterstained without toning (first control), another is toned under the microscope until the unmyelinated fibers are definitely outlined (a matter of seconds) and then counterstained (second control), whereas the remaining ones are destained varying amounts and then toned to different intensities and finally counterstained. Such a procedure gives one two controls (counterstained, toned and counterstained) whereby the effects of the destaining and subsequent toning can be checked. Worthy of emphasis is the fact that sections of silvered material, prepared by the chromate method, can be successfully counterstained (Foley, '38) without toning in gold chloride.

The fact that over-impregnated preparations can be successfully destained is beautifully demonstrated by Duncan and Keyser ('38) in a recent quantitative study of the fibers and cells in the dorsal roots and ganglia of the cat. These authors point out that sections of tissue which are too heavily

impregnated can be faded with nitric acid, "thereby producing very clear and easily counted specimens from otherwise unsuitable material." Likewise the same authors emphasize the importance of counterstaining, thereby supporting the contentions of the writer (Foley, '36, '37, '38) that counterstaining is an almost indispensable adjunct in quantitative studies on peripheral nerves that contain considerable numbers of small fibers.

Also the chromate method is applicable to nervous tissue of lower animals. Dr. P. B. Armstrong of Syracuse University, has secured good results on the central nervous system of *Amblystoma* larvae with the following variation of the method: 1) fix 24 hours in the ice box; 2) wash several hours, or until color is no longer extracted, with 40% alcohol to which 15% pyridine has been added; 3) put into pure pyridine for 24 hours; 4) wash in many changes of distilled water for 24 to 36 hours; 5) silver for 3 days in 2.0% aqueous silver nitrate; 6) rinse 1 minute in distilled water; 7) reduce 6 hours in Ranson's pyro-formalin reducer; 8) dehydrate with alcohol and embed from butyl alcohol. The results secured by Doctor Armstrong are particularly significant when it is remembered that amphibian material is noteworthy for its refractory behavior to silver stains.

SUMMARY

The smallest nerve fibers can be adequately segregated and stained with clarity in blocks of nervous tissue from the central and peripheral nervous system if the blocks are: 1) fixed in an alcoholic solution of 1 to 2% potassium bichromate to which pyridine and ammonium hydroxide have been added; 2) treated with alcoholic and aqueous solutions of pyridine; 3) washed thoroughly with distilled water; 4) silvered with a 2.0% aqueous solution of silver nitrate; 5) reduced with an aqueous pyro-formalin mixture and 6) dehydrated and embedded with a technique of recognized cytological value.

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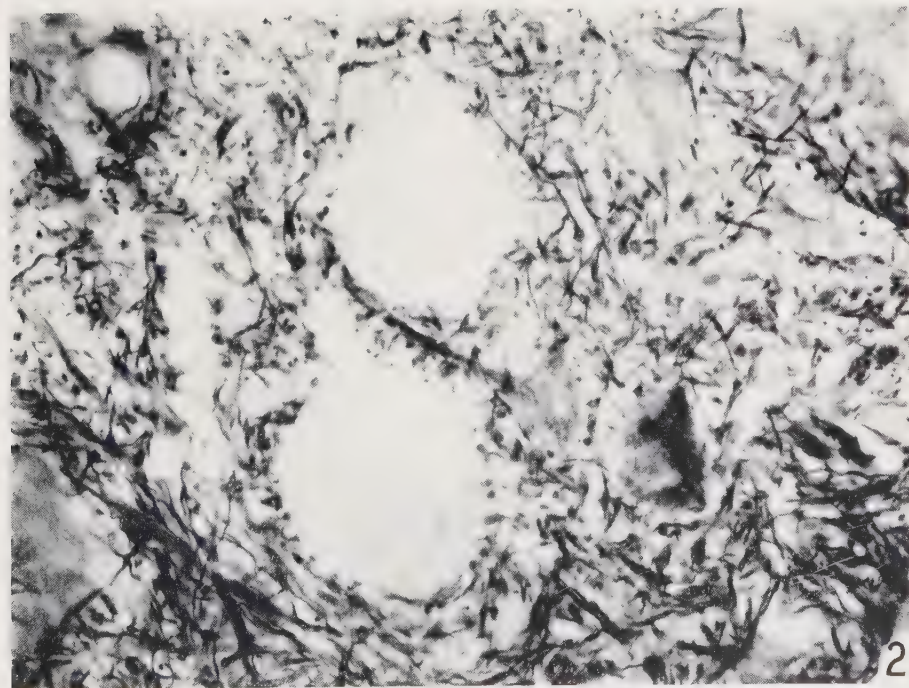
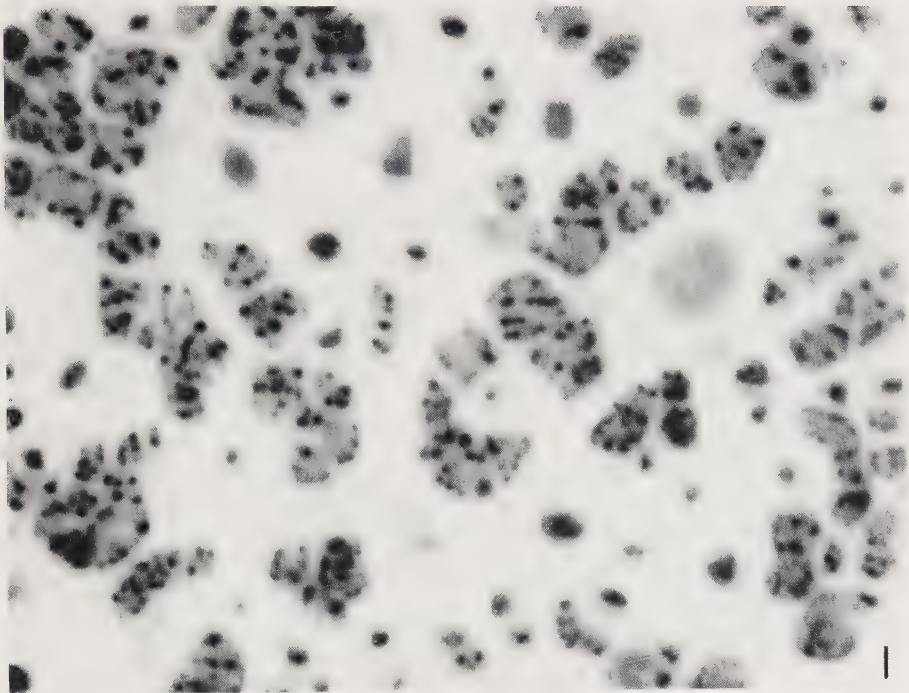
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PLATE 1

EXPLANATION OF FIGURES

1 An unretouched photomicrograph of a transverse section of the vagus nerve of the cat between the nodose and jugular ganglia ($\times 3000$ diameters). Attention is particularly directed to the groups of unmyelinated fibers embedded in their Schwannian matrix. Note their preservation of form and range in size. In addition it can be seen that neighboring fibers which are in sharp focus are adequately separated, not clumped.

2 An unretouched photomicrograph of an area of gray substance of the ventral horn of the spinal cord of the cat ($\times 600$ diameters). Observe the two large ventral horn cells near the center of the photograph. Worthy of emphasis is the fact that the neurofibrillae of these cells are not demonstrated; in other words, the technique is essentially a fiber rather than a neurofibrillar stain. A number of synaptic end bulbs and rings are in relation to these cells; some are evident in the figure.



THE CHARACTER AND DISTRIBUTION OF THE BLOOD CAPILLARIES

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SEVEN FIGURES

In previous communications (Zweifach, '37 a, '37 b), it was pointed out that two types of blood capillaries can be distinguished according to whether the vessels, along their entire length from arteriole to venule, are either partially or completely denuded of muscular elements. The architecture of the capillary system is such that the two types map out characteristic anatomical units, the arrangement of which is an important factor for the regulation of the local distribution of blood. The present paper represents a further contribution to the existence and functional significance of these two types of vessels and of their mode of branching.

MATERIAL AND METHODS

The living vessels were observed in the mesentery, tongue, skin and intestinal wall of the frog; also in the mesentery and ear of the white mouse. In addition, micromanipulative operations were performed on vessels of the mesentery in the mouse and frog. Fixed and stained preparations of the above tissues and of the omentum of the cat were also studied.

Details of the micromanipulative technique, as modified for the study of living blood capillaries in the frog and mouse, have been described (Zweifach, '37 a, '37 b). In brief, an intestinal loop was withdrawn and placed around a glass ring cemented over an opening in the roof of a horseshoe-shaped moist chamber. The tissue was covered with a glass

slip to whose undersurface it adhered and was kept irrigated with Ringer's solution. Mechanically controlled microneedles were brought up from below and inserted through the naked mesothelial layer into direct contact with the blood vessels of the mesentery (fig. 1).

The degree and rapidity of coloration of perivascular tissue by Congo red was used as an index of capillary permeability.

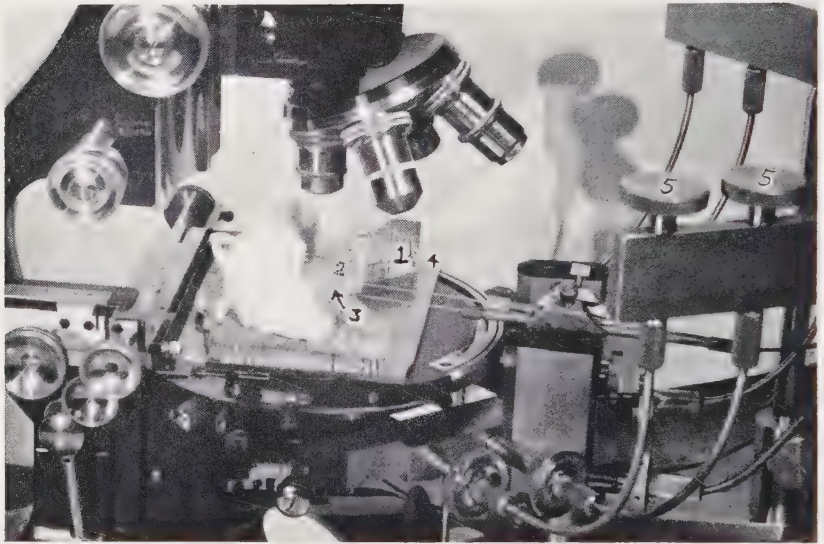


Fig. 1 Equipment for micromanipulation of capillary vessels in mesentery of mouse. 1, cork wall of moist chamber, with glass base (4), open at front for insertion of operating needles. 2, cover slip resting over exposed mesentery loop drawn over a glass ring on top of moist chamber and vaguely discernible (3 with arrow) through roofing cover slip. 5, 5, two of six control screws (others not visible in photograph).

Under normal conditions, no rapid coloring of the tissue about a capillary could be detected, when 1 to 2 cc. of a 2% solution of Congo red in Ringers was injected directly into the blood stream by way of the ventricle of the heart. However, with a change in the permeability of the capillary wall, the dye would seep through more rapidly into the tissue.

Often it was necessary to determine the number of cells that make up the wall of a particular capillary. A 5% to 10% silver nitrate solution was introduced with a micropipette and sprayed directly onto the capillary wall. This stained the cement substance between contiguous endothelial cells without impeding the circulation through that vessel.

I wish to thank Prof. Robert Chambers for his continued interest and cooperation throughout this work.

RESULTS

Because the concept of two types of capillaries is a new and important one, additional data together with photographic evidence is being presented in this paper.

Not all of the capillaries are functionally active at the same time or to the same degree. However, certain of the capillaries continue to convey an active flow of blood, even when the tissues are in a resting or anemic state. These latter vessels are main central highways for the continuous transport of the blood. A vigorous circulation always courses through them because, as direct extensions of the arterial vessels, they offer the least resistance to the forward propulsion of blood. The arteriolar pathway, therefore, not only distributes side branches but also continues through without interruption to the venule. This continuous trunk may be divided for descriptive purposes into three portions, the terminal arteriole or precapillary, the capillary, and the venule (fig. 2). The capillary portion of the trunk, in contrast to the other, less constantly used capillary side channels, at all times connects the arterioles and venules and was designated in a previous article ('37 a), the arteriolo-venular or a-v bridge.

The contractile tunic of the small arterioles, being continued along the a-v bridge as a series of modified muscle elements, the bridge may be regarded as a 'muscular capillary.' The muscle cells progressively thin out and become increasingly branched in the longer a-v bridges so that the cells become

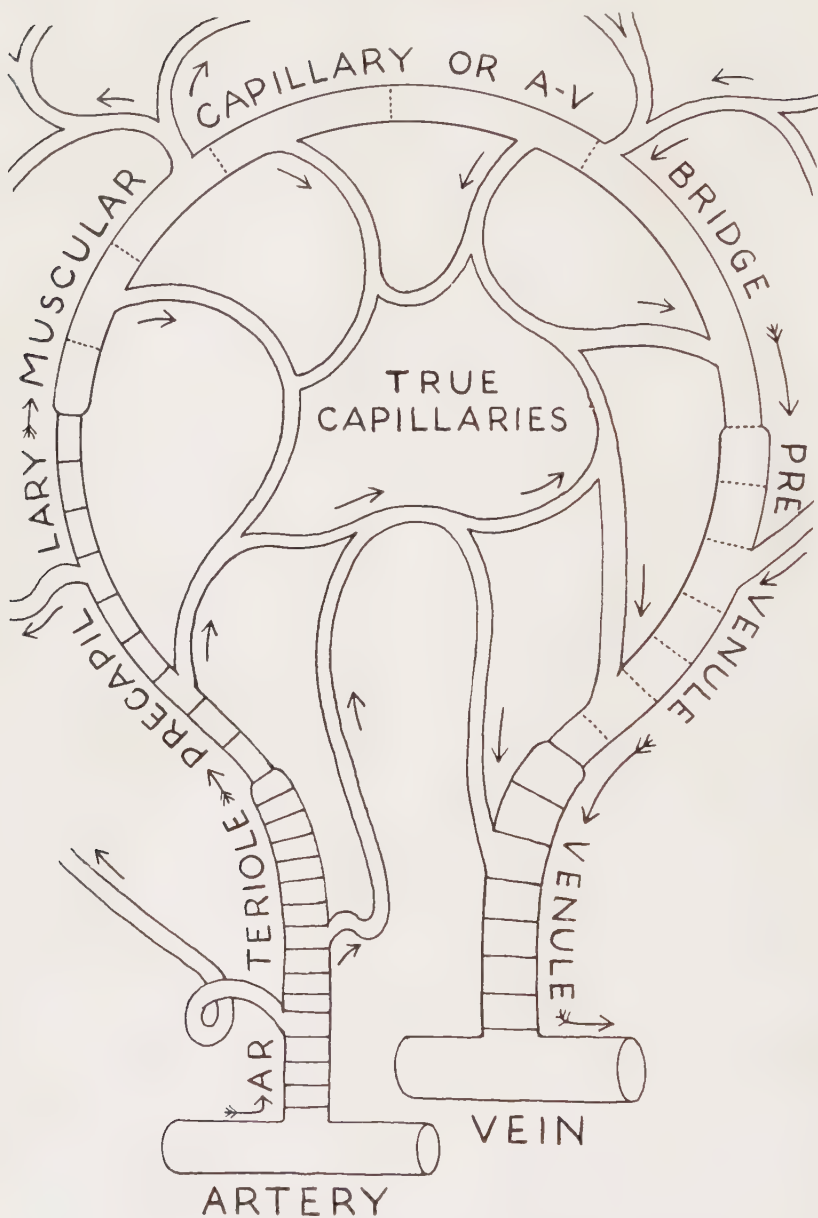


Fig. 2 Diagrammatic representation of capillary bed. Subdivisions of continuous central trunk are labeled and manner of branching of true capillaries is indicated. Typical smooth muscle cells are shown as solid cross lines, branched muscle elements by dotted cross lines.

indistinguishable from other adventitial elements in the distal parts of the vessels. Careful measurements showed that the a-v bridges are somewhat wider than true capillaries, being 12 to 16 μ in diameter.

The a-v capillary is to be considered as a central or focal pathway from which the remainder of the capillary vessels are distributed as side channels (fig. 3). These offshoots are purely endothelial in character and may be termed 'non-muscular or true capillaries.' The true capillaries anastomose freely with one another and finally rejoin the distal continuation of the a-v channel as it continues toward the venous end.

The two types of capillaries are more difficult to distinguish in fixed preparations because the most striking feature of identification is a functional one. A-v bridges in fixed normal tissues were characterized by containing a greater accumulation of blood cells than true capillaries.

Pattern in different forms and tissues

The number of permanently open capillaries, the a-v bridges, varies with different tissues according to the basal level of tissue activity. In addition, the ratio between the number of vessels of the muscular type as compared to that of the non-muscular type varies with each tissue. This depends upon the degree of fluctuation in activity which that tissue normally exhibits. In the skin, both in the frog and ear of the mouse, which may be regarded as having a constant level of activity, almost all of the minute vessels extending to the epithelial surface were found to consist of the a-v bridge type. In the nictitating membrane the two types of capillaries were seen in about equal numbers; in the mesentery the true capillaries were several times more numerous than a-v vessels; in striated muscle, where extreme fluctuations in nutritive demands occur, the a-v capillaries were relatively infrequent as compared to their abrupt capillary side branches which made up 75% to 90% of the vessels.



Fig.3 Arteriole-venular bridge shown through its entire course from its arterial to its venous end. Note offshoots constituting the true capillaries. (Reconstructed by assembling from a motion picture film.) Low power.

Angle of capillary branching and its significance

An interesting feature of the capillary pattern is the characteristic manner in which the successive segments of the central vascular trunk distribute their capillary offshoots. There appears to be a direct correlation between the intensity of blood flow in the parent trunk and the manner in which the capillary offshoot leaves the central channel. At the arterial end, the offshoots were found to come off abruptly, curve sharply backward and continue for some distance in a contorted or spiral course (fig. 4 a). Further downstream, where the muscular coat begins to be scattered, the capillary offshoots come off at right angles and then take a sharp backward course (fig. 4 b). The flow of blood in the above-mentioned offshoots is suddenly and markedly slowed. This slowing can readily be ascribed to the peculiar angle of branching. As the blood continued through the central trunk toward the venous outlet, a gradual diminution in the rate of flow develops and is paralleled by the less and less acute angles at which the successive capillary branches leave the a-v vessel (fig. 4 c, d). The most distal portions became progressively a receiving channel and are joined by tributaries that approach the vessel at a gentle slope and drain into it (fig. 4 e).

The abrupt side branches of the arterioles possessed muscle cells about their proximal curved portion, but not elsewhere along the offshoot. This portion, therefore, can be considered as a precapillary, the muscle elements of which are in direct continuity with those of the parent arteriole. At the point of exit of the capillary branch, the wall of the arteriole was usually reenforced by a grouping of muscle cells about that side of the vessel where the branch leaves it.

*Endothelial valves at capillary exit on arterial portion
of a-v trunk*

A valve-like fold of endothelium is present at the point of capillary exit in those regions where the capillary offshoot leaves the arteriole and a-v vessels in a sharp backward angle.

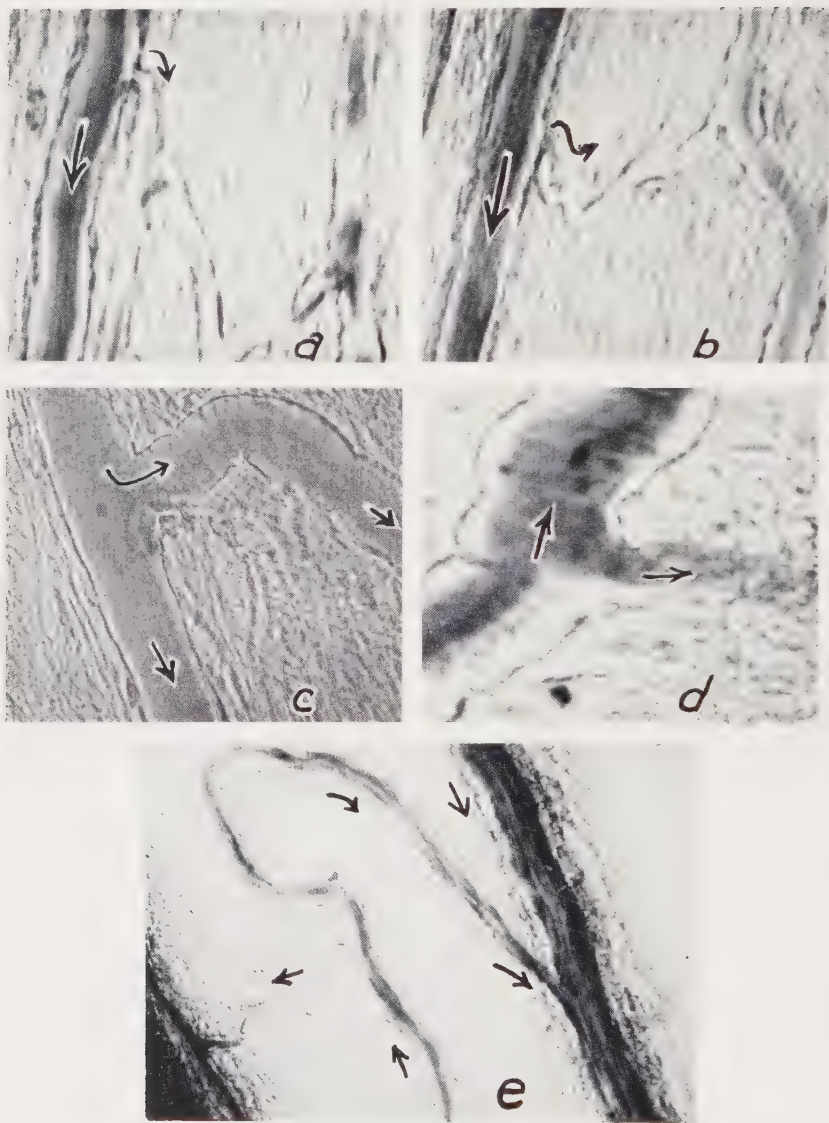


Fig. 4 Capillary offshoots from successive portions of the a-v bridge to illustrate the progressive change in angle of branching from (a, b) close to arteriolar source, through (c, d) in mid a-v region, to (e) where vessels fuse at a gentle slope to form a venule. a, b, e, low power; c, d, medium power. Mesentery of frog.

Two semi-rigid folds protrude into the lumen so that red cells, as they are swept by, are caught on the obstruction (fig. 5). The folds behave as an 'endothelial sphincter.' Movements of the a-v wall occur during periods of extended increase or decrease in blood flow through the vessel, and alter the degree to which the endothelial folds obstruct the orifice into the capillary offshoot. During a-v dilatation, the apposed, free edges of the endothelial folds were drawn further apart. This effected a wider passage for the entrance of blood into the capillary offshoot. The folds of the endothelial sphincter become more closely approximated when the parent trunk

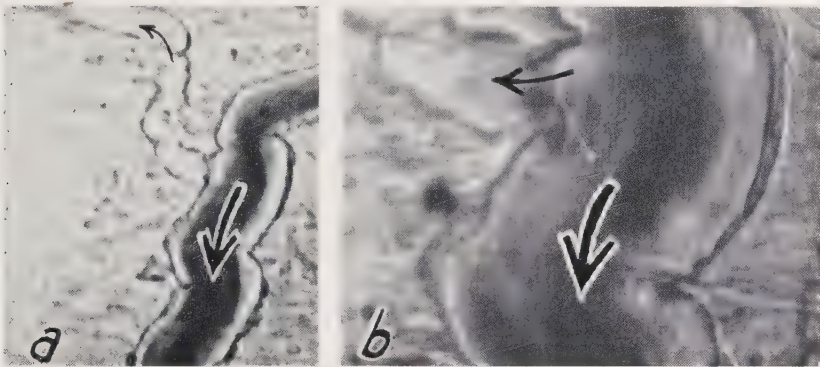


Fig. 5 Endothelial valves at point of exit of capillary offshoot on arterial end of a-v channel. a, low power; b, high power. Mesentery of mouse.

partially contracts and often the passage of blood cells into the capillary branch is completely prevented. In arterioles the partial closure was aided by muscle cells about the point of capillary exit.

Blood flow through capillaries

Freshly exposed tissues which have been disturbed as little as possible by handling tend to have a pale anemic color. Under such conditions, which may be considered as representative of the resting or basal level of the tissue, the circulation through the capillary bed occurred chiefly through the

a-v bridges. The true capillaries were either collapsed or simply devoid of circulating blood cells.

No active increase in capillary caliber was seen in anemic tissues so long as no blood circulated through that vessel. Expansion of the a-v circulation into the empty true capillaries was effected experimentally in a number of ways. The flow of blood was diverted from the a-v vessel into the capillary branch by pinching off the parent vessel just below the point of exit of a capillary offshoot. A similar effect was obtained by repeated compression of the a-v bridge at the point where it was joined by an empty capillary. Alternate compression and release invoked a suction effect that drew a pulsating column of blood in from the arterial end. Thirdly, when the tissue adjacent to an empty capillary was mechanically irritated for 1 to 2 minutes with a microneedle, a slow trickle of fluid and subsequently of blood cells was initiated into the capillary.

The lumen of the narrowed capillary opens in a series of stages. A partial increase in width appeared first with a seepage of fluid into the capillary. No further opening of the lumen occurred if at this stage the connected arteriole was compressed so that no blood could enter into the capillary. In the undisturbed experiment, however, a number of red cells were deformed and squeezed into the contracted capillary. The distorted cells bobbed back and forth and, as they did so, the portion of the capillary tube, with which they had made direct contact during each pulsation, gradually opened up. This type of sporadic flow continued until the vessel as a whole was wide enough for the red cells to pass through without being excessively deformed.

Capillary diameter

Capillaries differ in width not only through variations in distension, but by consisting of a different number of endothelial cells in cross section. One vessel observed in a completely dilated state may actually be narrower than a neighboring undistended capillary simply because the wall of the

first has fewer cells about its circumference. This was demonstrated by bringing the endothelial cell outlines into evidence with silver nitrate (10%), sprayed directly onto the vessel with a micropipette (fig. 6).

The most frequently used pathways, the a-v bridges, are the widest of the capillary vessels, with five to seven cells in cross section. The accessory side channels, the true capillaries, have but two to five cells about their circumference. Because of this discrepancy between two and five cells, the true capillaries show wide extremes in their normal width. After continued hyperemic blood flow, the larger of the true capillaries distend and approximate the diameter of the a-v vessels.

The true capillaries differ greatly in length. Short vessels, during the period in which they convey blood, have a nearly uniform diameter throughout their extent. In contrast, long capillaries become considerably wider on their venous side.

Long continued observations of undisturbed capillaries disclosed no sudden marked alterations in caliber. On the other hand, mechanical stimulation of the naked endothelial wall elicited, in both mammals and Amphibia, a definite localized thickening, wrinkling and indentation. The greatest endothelial response was obtained in the true naked capillaries. Responses were found to be more marked in capillaries of the frog than in the mouse. The indentation response becomes less and less evident as the endothelial tube is explored along its course toward the venous channels. A similar gradation in the magnitude of response is found in the various smooth muscle elements along the a-v channels. Perivascular smooth muscle cells in the arteriolar portions were most effective as contractile units. With a progressive loss in the typical spindle shape of these muscle cells along the a-v vessels, there was a corresponding decrease in their contractile effects. Contractile perivascular elements were conspicuously absent on true capillaries. With the transition to the small venule type (prevenule), fusiform smooth muscle ele-

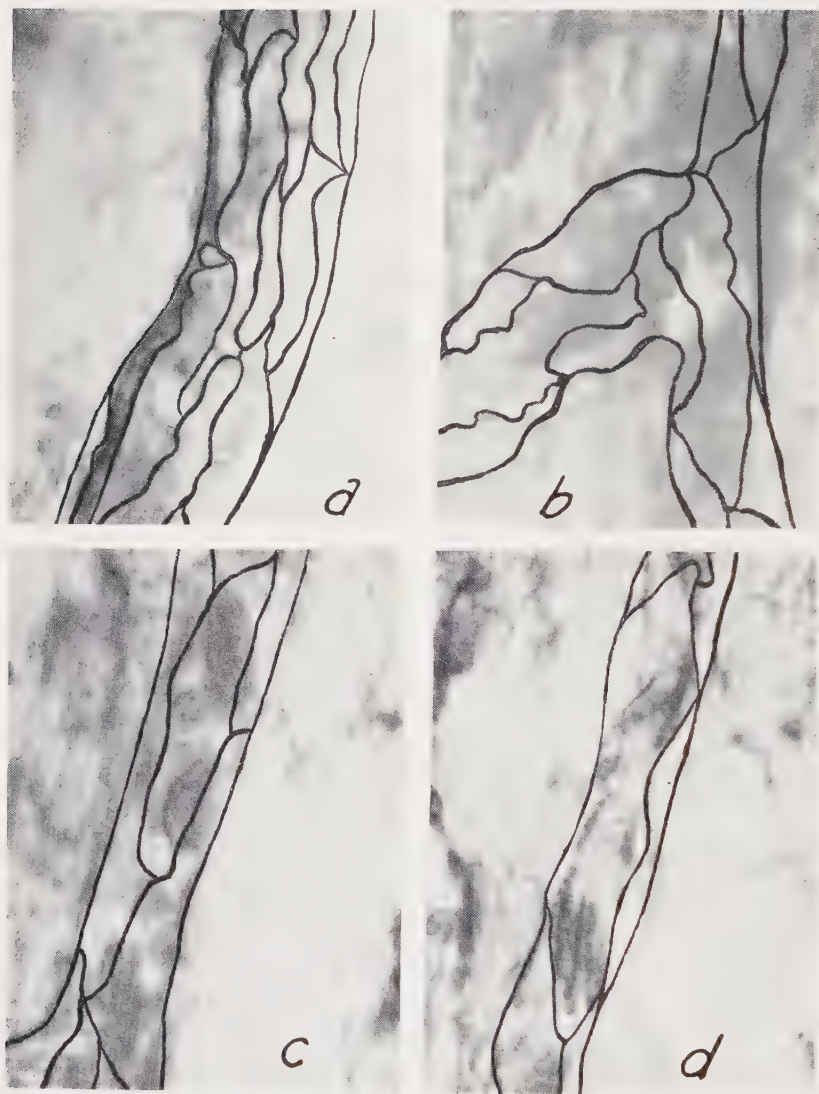


Fig. 6 Endothelial cell outlines demonstrated by silver nitrate sprayed on living capillary wall with micropipette. b, c, d are true capillaries; a is an a-v bridge. Medium power, mesentery of frog.

ments appeared again and were capable of indenting the underlying wall.

Influence of capillary tone

The normal true capillary possesses tone in that it behaves as a reversibly distensible tube. The presence of the tone must be ascribed to the endothelial cells that comprise its wall. Alterations in the tone (turgor) of the endothelium is one of the factors that influence capillary diameter. It was found that when the tissue was repeatedly irritated by mechanical prodding, the nearby capillary dilated although the blood pressure had not been changed. A similar dilatation was produced by re-routing venous blood so that it flowed in an opposite direction through the capillaries. This was done by compressing a venule with the side of a micro-needle just below the point of junction with a capillary vessel. Under this condition of temporary dilatation, the endothelium was found to be more pliable and less rigid than normal, and offered little resistance to distortion by the needle.

During the resting stage of activity, the true capillaries, both in the mouse and frog, maintained a relatively fixed diameter so long as blood continued to flow through them. Increased blood flow alone had little effect on capillary diameter. Likewise, loss of tone in vessels with no flow through them had no influence on capillary caliber. Vascular distension occurred only in capillaries which had blood circulating through them. Such evidence indicates that before the blood pressure had an effect on diameter, the endothelial wall previously must have been weakened by loss of its normal tone.

The removal of the factor responsible for the hyperemic flow was followed by a gradual slowing of the circulation through the a-v bridges and a slight narrowing of the vessel. In turn, the flow of blood through the capillary offshoots became sporadic and then gradually ceased. Those true capillaries, in which no blood was circulating and which were in good tone, narrowed over an extended period and were com-

pletely contracted within 15 to 40 minutes. Vessels whose tone was less marked remained open but, due to a change in the color of the lumen and vessel wall, gradually faded from view. It should be emphasized that capillaries with an active circulation were never seen to contract down so as to interrupt the flow of blood through them.

Local anemic and hyperemic states

The change from a resting to a more active vascular condition occurred spontaneously in a mesentery that had been exposed for about 30 to 80 minutes, and in the intact skin which had been unduly handled or warmed. Similar changes were induced by mild, prolonged local manipulation of the capillary bed and its surrounding tissue. In such cases a filling of the stagnant, true capillaries and a dilatation of those in the immediate vicinity of the focus of irritation occurred even when the pericapillary tissue alone was manipulated (fig. 7).

The outstanding vascular phenomena, that were involved in the change from an anemic to a hyperemic condition, occurred in the following sequence:

1. The blood cells became more closely packed and concentrated as they traversed the length of the a-v capillary and approached the venous end. The increased viscosity of the blood presented a higher venous resistance.

2. The normal capillary wall did not permit Congo red to pass out of the blood stream in quantities sufficient to stain the perivascular tissue. At this point, however, an increased outward passage of the dye was visible.

3. The circulation was temporarily slowed through the open a-v channels.

4. The pericapillary tissue became heavily stained by a more noticeable egression of Congo red from the blood stream.

5. The venous portion of the a-v bridges and small venules were the first to become somewhat distended.

6. Those arterioles that distributed blood to the capillaries in this area are dilated and, due to an increased propelling force, the capillaries became flushed and filled with blood.

7. Many new capillary side channels began to be opened.

8. Continued distension of the a-v wall widened the orifice between the endothelial folds interposed at the point of capillary exit, so that blood more easily entered the capillary side channels.

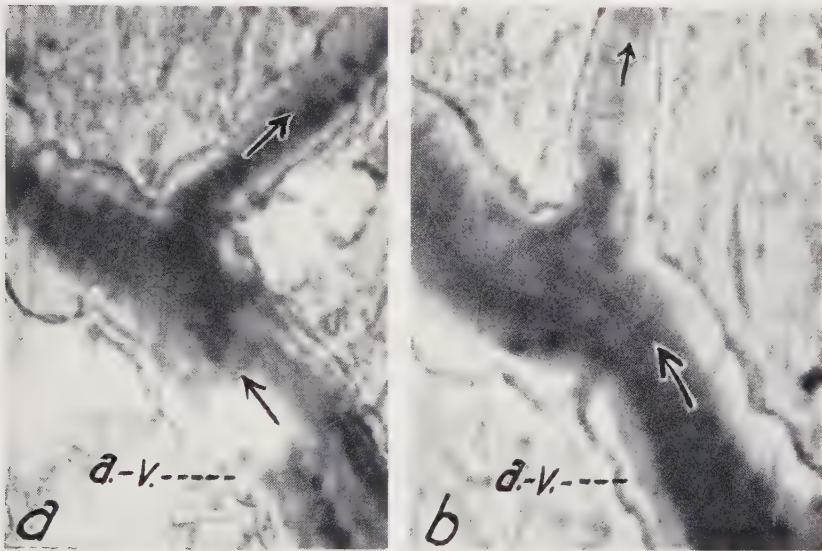


Fig. 7 Dilatation of a-v bridge and filling of true capillary branch following irritation of pericapillary tissue further distally along same vessel. a, before b, after manipulation. Medium power, mesentery of frog.

9. Finally, the continued rapid arteriolar flow introduced blood into all of the capillary channels.

An interesting detail here is the method whereby a circulation of blood was diverted into stagnant capillaries. When such a capillary was irritated, Congo red seeped through its wall indicating an increase in its permeability. This was followed by a slow movement of the entire column of blood until there was an active circulation through that vessel. One might therefore assume that, under normal conditions, an ac-

cumulation of metabolites about a stagnant capillary acts on the vessel wall by increasing its permeability. This in turn will be reflected by a movement of blood toward the region of increased permeability.

DISCUSSION

When the capillary bed is considered from an embryological viewpoint, the subdivision into two types of capillaries becomes apparent. Thoma (1893), Clark ('18), Chapman ('18), and Hughes ('35) have shown that in the embryo only those capillaries which had a vigorous flow of blood through them differentiated into arteries and veins. The other vessels either disappeared or persisted as capillaries. It is suggested that a similar type of differentiation may also occur in the capillary bed during its development. The extent of the transformation of the pericapillary elements would depend upon the intensity, type and duration of blood flow to which the vessel has been exposed. Vessels which were subjected to an active flow of long duration now possess specialized perivascular elements. Furthest advanced are the a-v bridges which are capable of acting as capillary vessels but are structurally more advanced than the undifferentiated true capillaries.

It is probable that even the adult capillary bed is not a fixed entity, its constituents being very labile structures. The nature of the perivascular elements may change with extended variations in the circulation through these small vessels. Clark and Clark ('35) followed in the rabbit's ear the development of muscular vessels out of undifferentiated endothelial tubes and described reversible changes in their distribution with variations in the blood flow through that tissue. The preponderance of a-v capillaries in tissues like the skin further demonstrates the effect of a blood flow, that is maintained at a certain level of intensity, upon the nature of the capillaries in that tissue.

The experiments of L. Hill ('21) can be offered as indirect evidence for the classification of capillaries into two groups.

He used the Roy and Graham-Brown apparatus for capillary pressure and found two groups of results. The gradual application of pressure interrupted the flow first through the side channels "but it continued through the most direct pathway connecting arterioles with venules, wherein the velocity was greatest to start with." Evidence like that of Kahn and Pollack ('31) and Fields ('36), in which two kinds of contractile response were obtained following electrical stimulation, likewise substantiates the existence of two types of capillaries.

Hughes ('37) found a direct relationship between the intensity of blood flow and the diameter of the embryonic capillaries. The stimulus of the blood flow acted to increase the number of endothelial cells that formed the capillary. It was therefore not surprising to find that the so-called normal diameter of adult true capillaries varied with different vessels, because of this fact alone. This makes it difficult to evaluate the comparative degree of dilatation of contraction of neighboring capillaries by simple observation.

The rate of blood flow also has a direct influence on the manner in which the side branches leave the central trunk. Roux (1895) and Thoma (1893) found that the angle at which branches leave the main trunk can be related to the fluid friction generated in such a system. Such a relationship was found along the central trunk from arteriole to venule in both the frog and mouse. The progressive slowing of blood flow is accompanied by a change in the manner and angle at which the capillary offshoots leave the arteriole and a-v vessels.

Most of the true capillaries are abrupt side branches and as such can be effectively isolated from the circulation through the true capillaries. Jacobi ('20) also found that a majority of the capillaries are at acute angles and contain no blood most of the time. The abrupt manner of branching introduces a set of endothelial obstructions or folds at the point of junction. These also act to cut off the true capillaries from the circulation. Klemensiewicz ('21) pointed out the

sphincter-like closure of the capillary junction with the precapillary during partial contraction of the parent trunk.

The central vascular trunk is featured by two possible contractile elements, endothelium and smooth muscle, whose distribution counterbalances one against the other. The terminal arterioles are narrower than the succeeding capillaries because of the presence of well-developed muscle cells in their walls. In this portion of the vascular tree, the contractile effect of the endothelium is relatively unimportant. Endothelium is contractile but this is effective only within certain limits. Where the blood pressure is high, as in small arterioles and precapillaries, endothelium is supplemented by well-developed smooth muscle cells. Further distally, with a diminution in intensity of blood flow and a decline in the number and character of the pericapillary smooth muscle elements, the endothelium becomes a more potent factor. Finally, in the true capillary side branches the endothelium remains as the sole contractile element.

The changes in capillary diameter that do occur are slow and passive. What is the mechanism underlying these gradual changes in diameter? It appears that three factors, acting separately or together are concerned: tone of endothelium, inherent elasticity of endothelium, and intercapillary pressure. The relative amount of tone was interpreted and measured by the reaction of the endothelium to prodding and manipulation. It was thus shown that endothelial tone fluctuates with alterations in the activity of the tissues and the resulting accumulation of metabolites in that area. Clark and Clark ('35) found a similar change in endothelial 'elasticity' with dilatation of the capillaries.

The interplay of two opposing factors, intracapillary pressure and endothelial tone, produced varying results depending upon changes in either or both of these factors. Dilatation was the result of poor tone accompanied by an increased or maintained intracapillary pressure. Closure of the capillary lumen was a result of ischemia following a period of active circulation. The a-v blood flow is sufficient to nourish the

tissues during the ischemic state. The presence of good tone and diminished intracapillary pressure in the empty true capillaries resulted in a gradual narrowing of these vessels over a period of 15 to 30 minutes. Similar, drawn out contractions are reported by Sandison ('32), and Clark and Clark ('35) in the rabbit's ear. A somewhat analogous situation is reported by Rous and Gilding ('29) who found that after ischemia induced by bleeding, the deprivation of blood invokes a local vaso-constriction.

Interpretation of mechanism of hyperemic capillary flow

When blood is pumped under low pressure into the elastic capillary, the blood will be propelled in a forward direction so long as the resistance ahead of the tube is not excessively high. When the pressure is raised and an increased amount of blood is pumped into that vessel, there will be a distinct tendency for the circulating fluid to distend that tube. Normally, the pressure in the small arterioles is maintained at a level just sufficient to overcome the resistance of the vascular tree ahead (in the venous end of a-v vessels and pre-venules). This main channel is used exclusively so long as the traffic ahead is not too congested. With increased venous congestion, the side pathways are opened and the flow is redistributed.

Two effects of local disturbances, such as arise through mechanical irritation, were seen during the change into a hyperemic circulation. An increased capillary permeability develops soon after the onset of the irritation and results in a concentration of the blood elements as they traverse the vessel toward the venous end. Within 3 to 5 seconds a 'reflex' dilatation of the feeding arteriole ensues. Whether the increased venous resistance or the tissue irritation act separately or together to produce the arteriolar dilatation is not clear.

The blood, which now enters the a-v bridge under an increased propelling force, fills the vessel to capacity and not only distends it, but, in addition, spurts out through the weak

points in its wall, the numerous orifices into the capillary offshoots. These factors, combined with the concurrent movement apart of the endothelial folds at the entrance into the capillary offshoots, enhance the flow of blood into the hitherto stagnant, subsidiary channels, the true capillaries.

The reverse occurred as follows: With a diminution in the resistance of the venous vessels, the arterial pressure was again free to be translated into the forward propulsion of the blood. The removal of the cause of irritation was rapidly followed by a diminished arteriolar flow. In turn, the distended elastic endothelium of the a-v vessels slowly thickened and, as the vessel became narrowed, the endothelial folds at the entrance to the capillary side channels were more closely approximated. The closure of these points of exit cut off the circulation from the true capillaries. Within 5 to 6 minutes only the a-v bridges contain a continuous flow of blood and the circulation is again of the basal or resting type.

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EVIDENCE OF AN EPITHELIAL LINING IN THE LABYRINTH OF THE AVIAN LUNG

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TWO PLATES (TWELVE FIGURES)

A recent paper (Bremer, '38) was devoted to the description of two methods for the detection of protoplasmic films too thin to be ordinarily visible through the microscope and to the demonstration of such films in the mammalian fat cell, renal glomerulus, and pulmonary alveolus. In the two latter cases the films appeared as broad expansions from thicker nucleated cell bodies; they covered the free surfaces of the blood capillaries and formed most of the lining of the capsular or alveolar cavities. One method is that used by Wassermann ('37) for fat cells and by Bensley and Bensley ('30) in somewhat different form for the renal glomerulus, and consists in actually swelling the protoplasm by hypotonic solutions until a film of ultramicroscopic thinness becomes microscopically visible; the other depends on the ability of certain types of protoplasm, even when in the form of a thin film, to take up vital dyes after 'chronic vital staining,' i.e., prolonged subcutaneous injections of the dye followed by a rest period. By combining these two methods the dye granules can be seen in the swollen protoplasm, thus proving that they are not merely adherent to the surface.

In the present paper the same two methods are employed in the study of the bird's lung. Here, as with the mammalian lung, conflicting opinions are expressed, as to the presence or absence of definitely lined air spaces, as to whether or not the air 'breaks through' into the tissue spaces and bathes

directly the blood capillaries there encountered. An analysis of the literature on this question has been given by Bargmann ('35) and need not be repeated here. It may, however, be useful to review the anatomy of the bird's lung to render more intelligible the illustrations here used.

As is well known, the avian bronchi divide into many branches, which together form the more compact portion or true lung, and then continue further growth to expand in the cervical and abdominal regions into the various air sacs. This process is described by Juillet ('12) and by Loey and Larsell ('16 b), who also noted that from these sacs a second growth leads to the formation of recurrent bronchi which re-enter the lung proper and there anastomose with the original bronchial branches. Anastomosis seems to be inherent in the avian apparatus. Within the lung further subdivision of bronchial branches occurs, and also further anastomosis between branches, which now are called parabronchi of various orders (lung pipes, entobronchi, ectobronchi, etc.). From these connecting tubes innumerable small radiating side pockets, called vestibules, are developed, which divide once or twice and communicate with the labyrinth, where the air comes in close relation with the blood capillaries. The labyrinth surrounding the individual parabronchi occupies an area roughly hexagonal in transverse section because of the mutual pressure of the more or less parallel tubes. The minute histological character of the labyrinth is the point in question.

The arrangement of a single parabronchus is shown in diagram in figure 1. The mouths of the vestibules are guarded by bundles of smooth muscle lying directly beneath the entodermal epithelium and forming together a muscular lattice or network running along the tube. The amount of muscle varies with the different orders of parabronchi and also with the type of bird studied. The epithelium may be cuboidal or flattened, as shown in Bargmann's figures 11 and 12. From the sides of the vestibules as well as from their outer ends the air may pass to the labyrinth, where the layer of epithelium is apparently lost. In the chick the air spaces

or 'air capillaries' are of about the same size as the blood capillaries with which they intermingle. The arteries enter from the connective tissue septa bounding the hexagonal areas and soon branch to join the capillary net. The veins receive the capillaries for the most part at the central border of the net, and tend to run lengthwise immediately beneath the muscle bundles before turning outward to join the arteries in the septa. The air in the labyrinth is bounded grossly by the septa peripherally, and centrally by the walls of the vestibules, by the connective tissues underlying the muscle bundles, or by the outer walls of the veins.

Attempts to follow the development of the finer air passages in birds have given only conflicting results. Juillet writes that after many studies of embryos between the sixteenth day and hatching he was unable to follow the development of the pulmonary parenchyma. Locy and Larsell ('16 a) state that "By continuous growth these branches [of the vestibules] penetrate further into the tissue . . . and the air capillaries are soon formed on their extremities." They give no illustration bearing on this statement, and only a simplified diagram of the arrangement in the adult. Bargmann, on the other hand, shows by two drawings that the change which occurs in the lung just before hatching consists in the loosening of the connective tissue of the labyrinth region, the extreme thinning of the epithelium lining the vestibules, and the breaking through of the air into the tissue spaces. He could never convince himself of any sprouting of the epithelium; on the contrary he considers that the very thinness of the vestibular epithelium indicates its degeneration and opens the way for its perforation and almost complete disappearance.

The literature offers, to my knowledge, no reconstructions of the bird's lung at the critical stages, probably because of the intricacy of the labyrinth at that time. One reason for the appearance of intricacy was discovered by accident. In frozen sections of chick lungs examined under water, as will be described later, the view was obscured by the great number

of air bubbles, which are held more persistently in the small passages of the avian lung than in the larger alveoli of mammals. To avoid this air the lung of a chick embryo, killed before breathing had commenced, was prepared in the same way, yet again a multitude of minute bubbles was present. Their microscopic appearance and the application of Sudan III proved these latter to be fat droplets, not air bubbles. The avian lung, both epithelium and connective tissue, undergoes just before hatching a fatty degeneration (Bargmann's 'loosening'), and in this duplicates the action of the mammalian lung as described by Stewart ('23). In serial sections of fixed material, from which the fat has been removed, the circular holes in the labyrinth may represent either air capillaries or former fat droplets, and for this reason intelligible reconstruction of the air channels from sections treated with ordinary embryological stains is impossible. However, by the use of the connective tissue stains, which show fine outlines around air spaces and of course none for the fat vacuoles within cells, reconstructions can be made with a fair degree of assurance. Such a reconstruction, representing a cast model of the air spaces, is given in figure 2. It shows several vestibules connected with the main cavity of the parabronchus (above) and as extensions from them numerous finer passages, some ending blindly, some already anastomosing with others from the same or neighboring vestibules. In the specimen they interdigitate with a plexus of blood capillaries. The regularity of the finer air passages seems to indicate an ordered growth, not a 'breaking through' into formless tissue spaces.

In this method of growth by extension of the cavities and anastomosis around intervening blood vessels, the terminal bronchial branches of the bird's lung, both in the main portion and in the air sacs, act consistently. The air sacs represent a growth of closed epithelial tubes into loose mesenchyma, in which there are remarkably few blood vessels; when a vessel is encountered, the tube flows around it and anastomoses on the further side in much the same way as does the

entodermal outgrowth of the middle ear around the chain of bones in its path. The cervical sacs also anastomose with each other. In the lung proper the bird's 'air capillaries' are also a growth of closed tubes, but now into a highly vascularized connective tissue. By anastomoses of the growing tubes around the individual vessels and with each other the fine meshed labyrinth is produced.

The air sacs are lined by a readily demonstrable continuous layer of epithelial cells. In the labyrinth no continuous lining is ordinarily visible; the blood capillaries are apparently naked except for a fine reticular investment (Ogawa, '20), and for the scattered, widely separated cells recognized by Bargmann and others as 'Deckzellen.' Those authors who claim a complete epithelial lining for the avian labyrinth rely on 'analogy' (Campana, 1875; Fischer, '05), on the silver nitrate technique (Juillet, '12), or on the presence of short rows of cuboidal or flattened cells found either in normal lungs or after stimulation of some sort in the air spaces directly under the pleura (Seeman and Merkulow, '30). It is the purpose of this present paper to place the concept that the air is everywhere enclosed by an entodermal layer on a somewhat firmer basis. This can best be done, perhaps, by direct reference to the accompanying illustrations.

The avian lungs most commonly used heretofore in the minute study of the labyrinth have been those of rather small birds, such as the chick, canary or pigeon. In these the 'air capillaries' and blood capillaries are rightly described as of about equal size, about the diameter of a red corpuscle. In larger birds, however, the labyrinth offers a different picture. The air passages in the turkey, for instance, are much larger, and reconstruction from serial sections is for this reason more readily accomplished, as is shown in figure 3. This present reconstruction is of a region similar to that in the lower right corner of the diagram (fig. 1), bounded to the right and below by the septum between this and neighboring labyrinths and to the left by a vein running to the septum and receiving several capillary tributaries, here shown as cut just before

their entrance. The vestibules which connected directly with this part of the labyrinth are not included in the reconstruction, but would extend obliquely from the upper left corner of the drawing to open into the main lumen of the parabronchus.

The striking feature of this reconstruction is the absence of 'air capillaries.' There are no minute tubular passages; instead one sees larger cavities, bounded partly by blood capillaries and partly by thin walls, and connected with each other either by broad openings or by smaller holes through the walls, reminding one of the alveolar pores redescribed recently in mammals by Macklin ('34) and by Loosli ('38). In fact there is a strong resemblance between my drawing of the reconstructed turkey lung and Loosli's figure 8, from a thick section of the rabbit lung. The holes or pores are in no sense tubular, as the term 'air capillary' would imply, and merely indicate the tendency of neighboring air spaces to anastomose.

At the peripheral border of the labyrinth the air cavities come in contact with the loose tissue of the septa and bulge into it in a succession of bays, separated from each other by double walls. Here the air is in relation, not with blood vessels, but with tissue spaces, or occasionally with a small lymphatic vessel. Presumably a minor part of the exchange of gases in this lung takes place between the air and the tissue fluids or lymph. The blood capillaries are sometimes entirely surrounded by the air cavities, sometimes appear suspended by a fold of the peripheral wall, reminiscent of a mesentery. The walls show large areas without vessels; but it is probable that this feature is exaggerated because, since the vessels were opened before this lung was fixed, it is almost certain that many collapsed capillaries escaped notice, that the capillary net is in fact much richer than it is shown. Still, both at the periphery and within the labyrinth, it is obvious that the cavities, though widely connected, are here and there separated by definite walls.

When the lungs of other birds such as chick and pigeon are reexamined after the study of the larger bird's lung, the relations in them of the air spaces to the blood vessels and to the tissue spaces are more easily understood. The air spaces are much smaller and the vascular net more closely woven, but the walls between vessels can often be found, especially at the peripheral border of the labyrinth, though they are, of course, of smaller extent than in the coarser lung. Rounded air spaces between encircling vessels are numerous and more conspicuous because the unperforated walls are less extensive, but the two types of lungs are fundamentally alike, the differences being due to the varying sizes of the air spaces and the relative richness of the vascular net.

In the reconstruction I have included the nuclei found in the first two or three sections, omitting those in the deeper portions for fear of complicating the figure unduly. They are of two fairly distinct groups, the one dark and elongated, the other larger, paler and more rounded. These two types Bargmann recognizes as belonging to endothelial cells and 'Deckzellen' respectively. In a few instances the positions of the two types in this figure agree with this interpretation, but many of the larger nuclei, instead of showing the proper 'Deckzellen' relation with a capillary, seem to follow the contour of the wall of an air space, at some distance from any recognizable vessel.

The walls of the air spaces can be studied most readily at the peripheral border of the labyrinth, where they abut directly on tissue spaces, lymphatics or blood vessels. In sections stained by the azan method a connective tissue membrane is here evident, the thickness of the fibers varying in different regions. No lining epithelium is ordinarily visible. Rarely, however, a short row of cells is seen, as noted also by Seeman and Merkulow; such a row as found in the turkey lung is shown in figure 4. The air space abuts partly against a vein, partly against tissue spaces. In the former position, where the thick vein wall prevents gas exchange, the cell protoplasm is of appreciable thickness; against the tissue

spaces and blood capillaries, the layer is apparently absent, but might perhaps merely be reduced to an ultra-microscopic film.

Attempts to show the presence of such a film by demonstrating dye granules in it after chronic vital staining, as was done in the rat lung in my former paper, at first gave only negative results. Neither in the chick nor in the pigeon were the granules present. Yet in both of these birds phagocytosis in the lung of material introduced by the trachea has been reported by several authors. Evidently phagocytosis at the surface of the air cavities and storage of dyes given vitally need not go hand in hand. A previous similar failure to effect the storage of dyes in the cat and monkey, though it was readily accomplished in the white rat, made it obvious that different animals reacted differently in this respect, and suggested that the same might be true of birds. The goose proved to be desirable for the purpose. Its large size was reflected in the coarseness of its lung, with relatively large air cavities easy to investigate, and in the young goose chronic vital staining proved successful, as is shown in the remaining figures. In thin serial sections of the fixed lung (figs. 5 and 7) the trypan blue granules are to be seen along the border of the air spaces of the labyrinth. In figure 7 the granules are located in the recognizably thicker protoplasm surrounding the lower epithelial nucleus and also extend further as a row where the protoplasm cannot be seen. To the left they extend over a nucleated (avian) blood corpuscle; the endothelium of the capillary in which it lay is not noticeable, but the granules lie between the corpuscle and the air space.

Frozen sections of these lungs placed in hypotonic solutions for half an hour up to several hours show areas in which a swollen sheet of protoplasm occupies the same positions, lining the air spaces or covering the blood capillaries. To drive the air from the smaller cavities in these sections it is necessary to compress the sections under the distilled water, which can easily be done by placing them on a raised table (a stack of glass slides) in a deep dish of water and rolling them

lightly under a round glass rod; the air escapes as fine bubbles and the tissue is elastic enough to assume its original shape. In the absence of the disconcerting refraction caused by the air, the blue granules, as in the case of the mammalian lung, can be seen in the swollen protoplasm inside the basement connective tissue fibers (fig. 12) or covering a blood corpuscle within a capillary (fig. 11). The granules show restricted molecular motion, as compared with the much more violent motion of other free floating particles in the cavity, indicating that they are actually within the protoplasmic gel. They can also be found in the recognizably thick protoplasm of the epithelial cells of the vestibules (fig. 10), where they again cover a capillary when this approaches the air cavity, as frequently happens.

Some of these rather thick frozen sections were stained with haematoxylin and watery eosin, both hypotonic solutions, then rapidly dehydrated, cleared, and mounted in balsam. Some of the swelling effect has remained, and from such preparations figures 6 and 9 were drawn. In their three-dimensional effect they are similar to the drawing of the reconstruction of the turkey lung (fig. 3). The trypan blue granules can be seen lining the irregular air spaces both where vessels are absent, as in the delicate partition walls, and in the presence of capillaries. In figure 9, for instance, the arrow 'a' is in a narrow air space, 'b' in a capillary partly filled by a blood corpuscle. Between the two is the fine line of the border of the capillary and a more filmy material containing granules, which is interpreted as a portion of the epithelial film. Other similar instances can be made out. It is impossible always to tell to what nuclear territory the particular portion of film may belong, because of the complicated shape of the cells, but the presence of such a film lining the air cavities can hardly be doubted.

Study of these specimens brings out another point, which refers back to my former papers ('35, '38). The post natal growth of the mammalian lung is accomplished, not by the increase of the individual alveoli, but by the formation of

new structural units and the addition of new alveoli. This is marked by alveolar buds, in the form of minute tubular sprouts which push their way into the tissue spaces of the surrounding pleura or interlobular septa. Such sprouts, also described by Hilber ('34), were shown in great numbers in serial sections and in models of lungs of young rabbits, cats, and opossums. Their walls are extensions of the alveolar walls and show either apparently naked capillaries or cuboidal cells, often in mitosis. In the rat lung after chronic vital staining some of these cuboidal cells in a tubular sprout contained dye granules (fig. 7, Bremer, '38), indicating that they were of the same cell type as the definitely entodermal cells of the bronchioles.

In the bird the lung also increases in size as the individual grows larger, whether in the egg or after hatching. In this case the increase may be due to the lengthening of the various orders of the bronchial tubes, to the growth of new branches of these tubes, or to an enlargement of the labyrinth enclosing the tubes. The first two possibilities would be difficult to determine without careful reconstruction of large areas of lungs of various ages; this has not been attempted. That the labyrinth increases progressively in thickness is shown by the following measurements. From its central limit at the base of the muscle bundles to its peripheral limit at the septa, the labyrinth in chicks of 17 days incubation measures on the average 0.08 mm.; at the twentieth day of incubation, 0.10 mm.; and in chicks 7 weeks after hatching 0.17 mm. It is obvious that the labyrinth is increasing in volume, but the average size of the air spaces is not appreciably larger. In the reconstruction of the chick before hatching (fig. 2) growth consists in the protrusion from the vestibules of narrow rounded sprouts, which push into the tissue spaces; some of them have already anastomosed with similar sprouts from the same or neighboring vestibules. In the young turkey lung (fig. 3, just above 'x') and in the young goose lung (fig. 8) similar sprouts can be seen. The first occupies only one section in the 5 μ series, the second lies within the thick-

ness of a single frozen section. The sprouts found in fixed tissues show no recognizable wall except the reticular or connective tissue basement membrane, while that seen in the swollen goose lung, after chronic vital staining, shows on one side a thickened film containing dye granules. It is probable that this sprout is composed of two cellular elements, only one of which stored the dye and responded to the hypotonic solution by swelling to visibility. Except for the fact that in the avian labyrinth no cuboidal cell bodies are present in connection with the thin films covering blood capillaries, as in the young mammalian lung, and that therefore no mitotic figures are seen, the sprouts in both cases are similar structures, hollow, tubular, and apparently forcing their way into the tissue spaces.

The urge of protoplasm to spread out over surfaces has been noted by W. H. Lewis and many others who study cells in tissue cultures. Especially interesting in the present case is the observation of Schopper ('32). He found that serosa cells in cultures always seek to cover surfaces; they may liquefy the plasma medium around them and spread out to cover these spaces. Often, as the spaces fuse, the cells are left as long bridges. Similar action at the borders of the labyrinth of tubular sprouts closed at the end, would give pictures comparable to those shown in figures 6 and 9. It is not necessary to rely on air pressure from within to account for the growth, though in post natal mammals this probably does account for the expansion of the sprouts into alveoli; in the avian lung before hatching the sprouts are present before air enters the lungs. The growth force seems to lie in the inherent property of protoplasm to spread over surfaces.

In these specimens of bird lungs 'Deckzellen' are not recognized. They have been reported by Bargmann ('35) as visible after perfusion and with special stains, occurring in small numbers far apart with discrete branching processes radiating from the nucleated cell bodies. The processes rest on the capillaries, and often bridge the space between capillaries (compare his figs. 4, 5 and 6). This latter statement of

Bargmann's is important. If the processes of the 'Deckzellen' are considered merely as thickened portions or ribs of a continuous protoplasmic sheet, according to the concept of Zimmermann ('33) and Bensley and Bensley ('30), their positions as bridges between capillaries indicates the presence of a continuous wall to the air spaces, of which wall the capillaries form only a part; this is precisely the condition shown in the present studies.

In the avian labyrinth, as was also noted in the mammalian alveoli, many areas are encountered in which the capillaries appear naked or where, at the periphery, the air cavities seem directly bounded by connective tissue. The illustrations here given are of selected regions. Two facts should be borne in mind; that only a few cells of an epithelial sheet, such as that lining the vestibules, will store the dye after chronic vital staining, and that according to biochemical measurements, a film may swell to many times its former thickness and yet remain microscopically invisible. Those two facts may well explain the apparently naked areas. The drawings here presented show that in certain areas the air cavities are bounded by a continuous epithelial sheet, covering the blood capillaries and the connective tissue walls between them. This positive evidence should weigh heavily against the negative, since the latter can be plausibly explained.

SUMMARY

Methods previously employed in the study of mammalian lung have been utilized in investigating the avian lung. Reconstruction of the lung of the chick before hatching indicates an orderly growth of the air passages, not an irregular 'breaking through' into tissue spaces. The lungs of large birds, turkey and goose, show a labyrinth containing large interconnecting air cavities bounded partly by blood capillaries and partly by thin walls with a connective tissue base. By the use of hypotonic solutions a film of visible thickness can in certain regions be demonstrated lining the connective

tissue of the walls. After chronic vital staining (goose) dye granules may be found within this film. In young birds, as in young mammals, tubular growing sprouts composed of this film are present, indicating the continued orderly extension of films over surfaces. That a continuous lining is not everywhere demonstrable may be explained by the known excentricity of vital dyes and by the possible extreme thinness of the film, below visible range even though swollen, as is probably also the case with sheets of endothelium. The evidence indicates, but not conclusively, that in avian lungs the air is everywhere enclosed by an epithelial layer.

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PLATE 1

EXPLANATION OF FIGURES

1 Diagram of an avian parabronchus and surrounding labyrinth, cut transversely. Arteries in black, capillaries and veins gray. Muscle bundles encircle the mouths of the vestibules, which branch from the main lumen and open into the fine cavities between the capillaries of the vascular net.

2 Model of part of a parabronchial wall of a chick of 17 days incubation; cast of the air cavities in vestibules and labyrinth. Shows apparent budding from vestibules of tubular outgrowths, some ending blindly, some having anastomosed with others. Capillary net is found between the outgrowths. $\times$ circa 500.

3 Reconstruction of part of the labyrinth, lung of a 3-month turkey. The region is similar to that in lower right corner of the diagram (fig. 1), bounded at right and below by septum bordering labyrinth, and at left by a vein receiving capillary tributaries, here shown as cut just before their entrance. Vestibules are not included in the reconstruction. The cavities are large and interconnecting, sometimes like alveoli, with obvious walls. Nuclei of only the upper two or three sections are included, and are of two types: 'a' epithelial; 'b' endothelial. At 'x' the wall of a cavity is seen from inner surface. $\times$ circa 750.

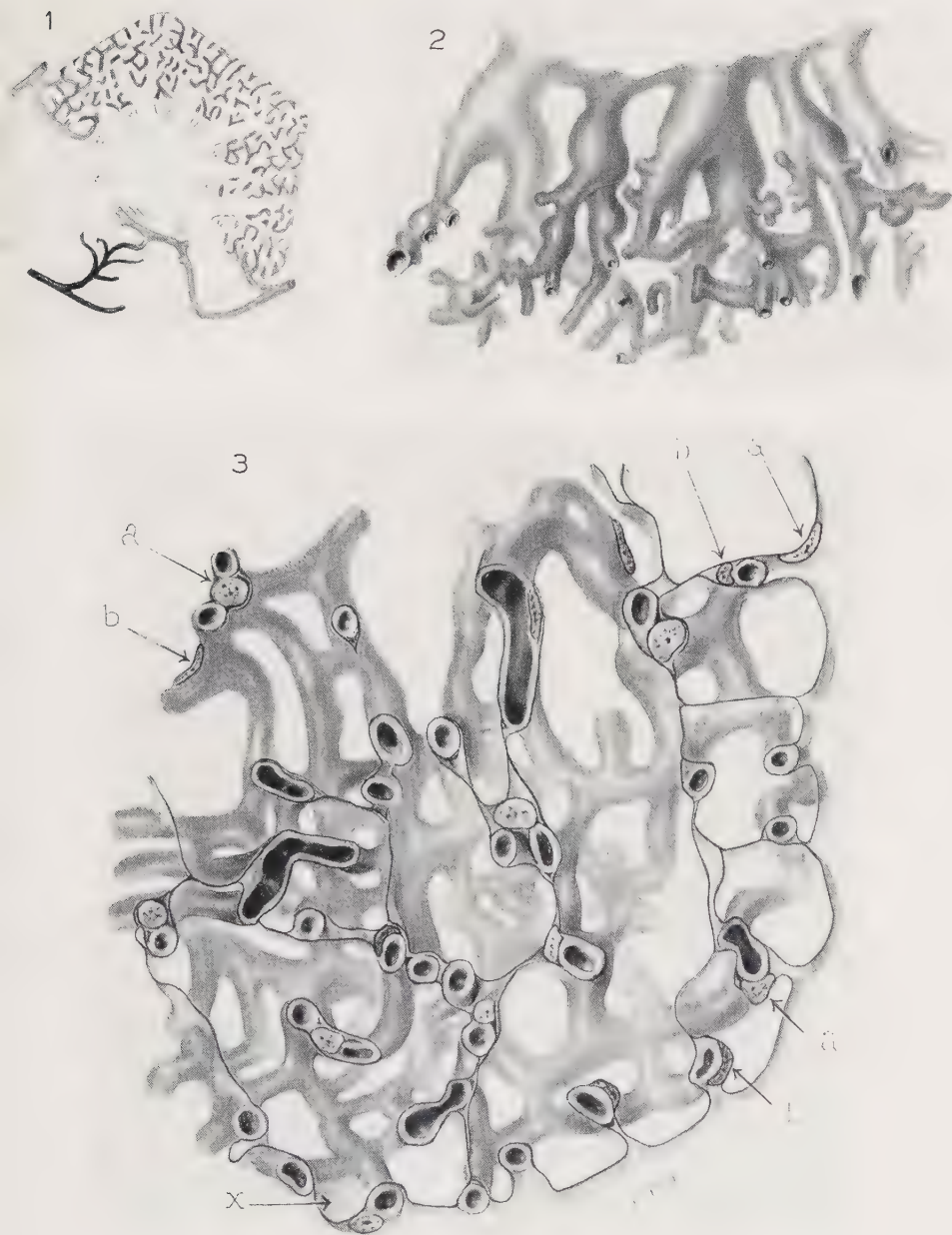


PLATE 2

EXPLANATION OF FIGURES

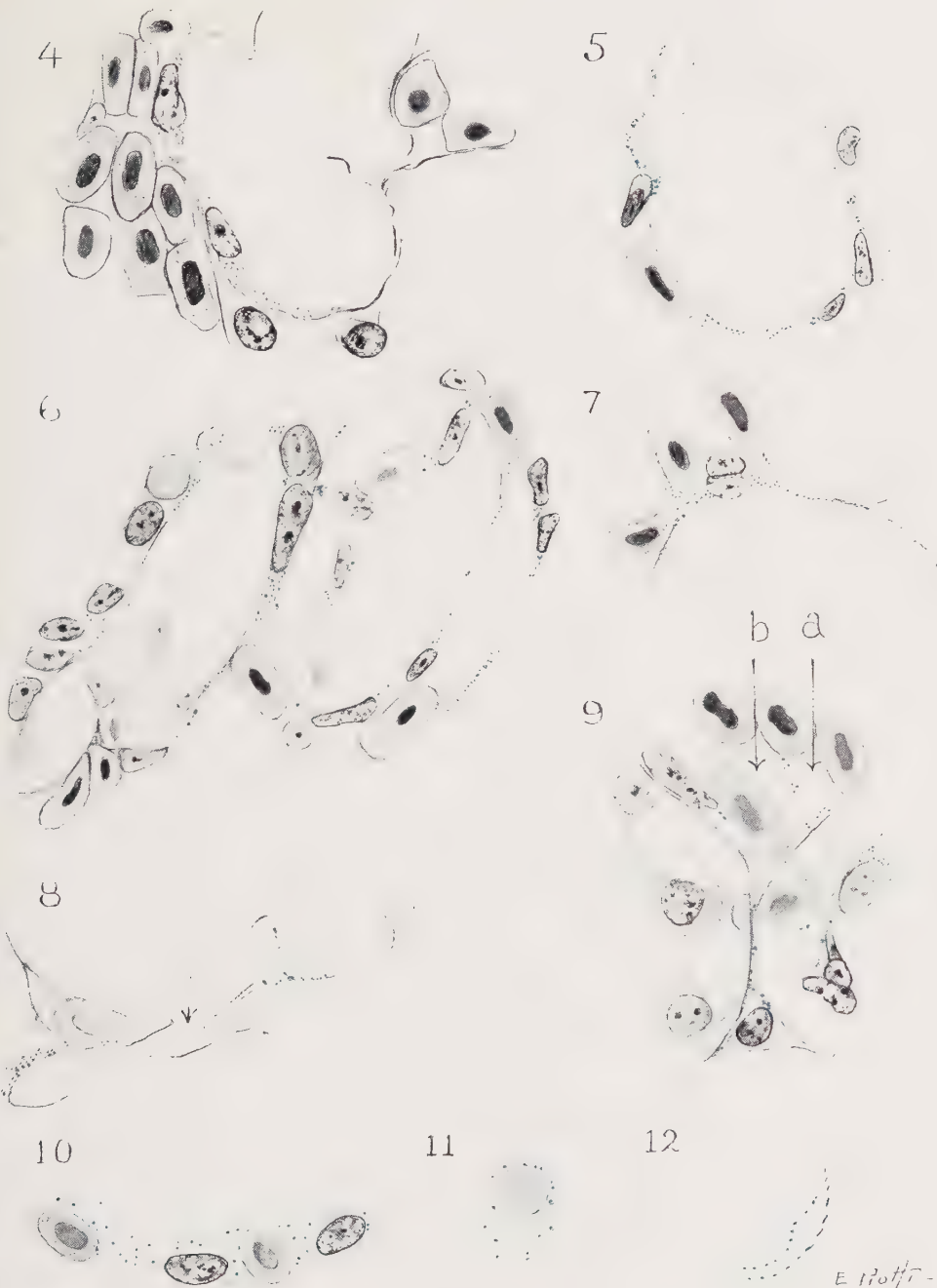
4 Turkey lung, air cavity at the peripheral border of the labyrinth, showing two epithelial cells where the wall rests on a vein or on connective tissue. No film is visible where the wall rests against blood capillary or tissue space.

5 and 7 Lung of goose, chronic vital staining, paraffin sections. The film lining the air cavities is revealed by the presence of rows of dye granules.

6 and 9 Lung of goose, chronic vital staining, frozen sections in distilled water, stained with haematoxylin and watery eosin, mounted rapidly. Inter-connecting air spaces, lined with slightly swollen protoplasmic film containing dye granules. 'a' points to an air cavity, 'b' to a blood capillary; between them is the dye-laden film. Other similar instances may be noted.

8 Lung of goose, chronic vital staining. At the periphery of the labyrinth an air cavity sends out a tubular sprout to surround a capillary. One side of the sprout has no visible film, the other shows a swollen dye-laden element.

10, 11 and 12 Lung of goose, chronic vital staining, frozen sections in hypotonic solutions, showing dye granules in swollen protoplasm. Figure 10, a vestibular wall (also stained with thionin for the nuclei); figure 11, film covering a projecting capillary, which contains a blood corpuscle; figure 12, film shown in relation to the refractive connective tissue fibers at the border of the labyrinth.



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BOOK REVIEW

SYNTHETISCHE MORPHOLOGIE DER NIERE DES MENSCHEN. Bau und Entwicklung dargestellt auf neuer Grundlage. By MARTIN HEIDENHAIN. Published by E. J. Brill, Leiden, 1937. xvi + 270 pages. 90 figures, 5 of them in color. Price 10 guilders; with linen covers, 12 guilders.

In the foreword Prof. Heidenhain makes a spirited plea for the 'synthetic' approach to morphology, an approach which he has championed vigorously for decades, and which has found expression in numerous publications, covering a broad field, by himself and by his students. He repeats his statements of 1899: that Schwann's theory is purely disintegrative; that it does not contain within itself the germ of a synthetic science, which could lead to an appreciation of basic natural laws, but teaches us, on a thousand different objects, a thousand times the same; that it will be the task of the future, through a synthetic theory of the tissues, to make whole again the animal body, which has been decomposed into its elements. Heidenhain puts forward the present work on the human kidney as an excellent example of the newer endeavors and their results, "for, apart from the confirmation of our general theory of forms, we have been able to represent anew almost the entire macroscopic anatomy and embryology of the kidney."

The book is divided into ten chapters. Chapter I includes a statement of the general problems of synthetic morphology, a brief review of earlier work upon the kidney, a section on terminology, the material and methods, and references to the literature (very brief). Heidenhain has re-examined the terminology of the kidney, and suggests many new terms (e.g. *ramus cranialis et caudalis* for major calyces, *plicae corticales* for renal columns, etc.). The material is extensive: serial sections of twenty-three fetal human kidneys, over-all length of fetuses 42 to 340 mm., kidney length 2.5 to 25 mm.

Chapter II deals with the macroscopic anatomy of the kidney. The conclusion is drawn that an organ shaped like the kidney cannot possibly develop in the same manner as any of the other large glands in the body (i.e., entirely by budding and sprouting). It is stressed that the *reniculi* of the composite kidney are analogs, not homologs, of the simple kidney (e.g. of the rabbit). Since the number of *ren-*

culi is at first small (only 6 in kidneys 2.5 to 3.5 mm. long), the surplus in the adult (8 to 12 on an average, even 16 or more) must arise through cleavage of those first present; a subsequent insertion of new renuli is theoretically impossible, and has never been observed.

Chapter III surveys briefly the histology (analytic, versus synthetic, morphology) of the kidney. Particular attention is paid to the structure and lobulation of the glomerulus. The appearance of long and short-loop nephrons is considered by Heidenhain to have no essential significance, but is simply a direct consequence of the arrangement of the nephrons in the given spatial relationships, the loops utilizing the spaces between the radially directed, dichotomously branching, collecting ducts. In the human nephron he finds the entire distal tubule (from the end of the thin segment) histologically uniform (with Stäbchen), without the differentiation of an indifferent Zwischenstück (without Stäbchen) such as occurs in the rabbit. In figure 11, purporting to illustrate the relationship of the ascending limb of Henle's loop to the renal corpuscle (of the same nephron), the point of apposition is opposite the vascular pole, i.e. in the neighborhood of the urinary pole. This is contrary to the facts; the ascending limb is invariably in close proximity to the vascular pole, and, more specifically, to the vas afferens (see Grafflin, *Archives of Pathology*, vol. 27, April, 1939). As a substitute for Peter's subdivision of the kidney into zones, Heidenhain suggests the simpler subdivision into zona corticalis, zona intermedia and zona basalis. He disagrees with von Möllendorff's conception of a vascular lobule of the kidney, versus the Traut lobule, built around the collecting ducts. He feels that the vascular arrangements are secondary to the building up of the epithelial formations, and that von Möllendorff has neglected to take into account the renal columns, comprising a large part of the kidney substance, in which the course of the vessels is quite irregular.

Chapter IV deals with the beginnings of kidney development. In the kidney of 3 mm. length, the ureteral tree has already developed, by budding and sprouting, about four orders of branches. The further increase in the collecting ducts takes place by cleavage, which begins peripherally and proceeds centrally. The 3 mm. kidney is already bean-shaped, and all of the end-branchings of the ureteral tree reach the kidney surface. The biological significance of the duct cleavages, upon which in turn the spatial arrangement of the nephrons is directly dependent, becomes understandable when we realize that, from the 3 mm. stage up to birth, the thickness of the cortex increases only fourfold (from 0.5 to 2 mm.) while the kidney surface increases about 275-fold (if we include the renal columns this figure would be at least doubled).

Chapter V discusses the multiplication of the collecting ducts through cleavage and the immediate consequences thereof. In the human kidney cleavage of the collecting ducts is a very extensive phenomenon, and is of extraordinary consequence for the architecture of the organ. Complete cleavage is frequently preceded by a separation-fold, which develops from a forked branching in the form of a double epithelial lamella, without basement membrane. Cleavages begin in the 3 to 3.5 mm. kidney; in the 5.5 mm. kidney they are in full progress; in the 11-12 mm. kidney they are present in considerable numbers, and they are always still demonstrable in the 14 mm. kidney; by them the kidney is transformed to a new type, with a radiate structure. The so-called ampullae never have the form or the histological character of apical buds, such as are found in the salivary glands and the growing lung.

Chapter VI is concerned with the apical growth of the collecting ducts in subsequent development. Heidenhain discusses his differences with Peter, who maintains that the essential process in the multiplication of the collecting ducts is budding and sprouting (not cleavage); and refers these differences to the period of development studied (Heidenhain the first, Peter the second half of pregnancy), the methods used, and the approach to the problem. This chapter is confined to the 14 mm. kidney (4.5 month embryo), in which apical growth of the collecting ducts is prominent, and leads to the formation of the collecting duct tree as described by Peter.

Chapter VII deals with the origin of the calyces and papillae. Of primary importance here is the appearance and particular mode of origin of the so-called epithelial mantle. In general, following the formation of a primary papillary area (with pre-calyx), and with the continuing cleavage of the collecting ducts and the resulting limitation of space, the epithelial mantle appears at the base of the six primary renculi. Subsequently it undergoes cleavage, giving rise to the primary papilla. With the extension (corticalward) of this line of cleavage, the definitive form of the papilla is attained. The development of the epithelial mantle is clarified by numerous, very instructive illustrations.

Chapter VIII considers the collecting duct systems, sectors and renculi. The pyramids, which are not yet present in the 3 mm. kidney, develop as the result of the extensive cleavage of the collecting ducts, and consist at first of radially arranged bundles of collecting ducts. These bundles Heidenhain designates as 'primitive medullary rays,' since they extend from the region of the papilla to the kidney surface. The medullary rays with their accompanying nephrons form the sectors of the renculus. In the development of the nephron Heidenhain

describes, as a new discovery, a zone of active cell proliferation—located in the descending limb of the loop of Henle, just at the bend—which is mainly responsible for the lengthwise growth of the loops. The subdivision of the *renculi*, by cleavage, begins at the tip of the papilla and proceeds corticalward through the pyramids. The origin of the renal columns is discussed.

Chapter IX discusses the kidney as a whole and its ‘ascending’ organization. The growth of the kidney is analyzed by the use of a new method of structural formulas, by which the progressive reproduction, by division, of the *renculi* and their calyces is very clearly expressed. The origin of the pelvis is discussed. A branched pelvis does not exist in the early stages of development, and is a secondary formation which does not correspond to the branching of the original ureteral tree. Finally, it is emphasized that the number of fetal cortical lobules is, in the fully developed condition, far greater (maximum around 50) than the number of *renculi* (usually 8 to 12, seldom more). Therefore we must distinguish, in the surface relief of the kidney, *colliculi majores* or *renculares* and *colliculi minores* or *sectoriales*.

Chapter X is a general consideration of the results of the investigation in their relation to the theory of living forms. It has been stressed for decades by Heidenhain and his school that the growth and organization of organs are to be thought of as dependent not only upon the assimilation, growth and division of cells, but also upon the reproduction and multiplication of composite tissue systems of higher order. The present study of the development of the kidney, taken in conjunction with its adult structure, establishes the human kidney as the best and most complete example of synthetic morphology which has yet been discovered in man.

Heidenhain has given us, in this treatise, an extremely important body of new and interesting data upon the development of the human kidney, and his findings are worthy of the most careful study. The book is superbly printed, and the illustrations are excellent.

ALLAN L. GRAFFLIN

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SUPPLEMENT

AMERICAN SOCIETY OF ZOOLOGISTS

Officers for 1939

<i>President</i>	J. T. PATTERSON
<i>Vice-President</i>	H. B. GOODRICH
<i>Secretary</i>	E. G. BUTLER
<i>Treasurer</i>	T. C. NELSON

Executive Committee

(In addition to the four officers)

A. H. STURTEVANT.....	to serve until December, 1939
ROBERT W. HEGNER.....	to serve until December, 1940
W. C. ALLEE.....	to serve until December, 1941
F. L. HISAW.....	to serve until December, 1942
M. H. JACOBS.....	to serve until December, 1943

Representative of the Society in the Division of Biology and Agriculture of the National Research Council

To serve until July 1, 1941.....	LAURENCE IRVING
	A. C. REDFIELD, Alternate

Representatives of the Society on the Council of the American Association for the Advancement of Science

B. H. WILLIER

C. R. MOORE

Representatives of the Society on the Council of the Union of American Biological Societies

H. W. STUNKARD

L. H. SNYDER

Representatives of the Society on the Advisory Editorial Board of "Biological Abstracts"

H. S. PRATT AND G. K. NOBLE.....	<i>Taxonomy</i>
D. H. TENNENT.....	<i>General Embryology</i>
L. V. HEILBRUNN.....	<i>General Physiology</i>

Representative on the Executive Committee of the Commission for the Standardization of Biological Stains

S. I. KORNHAUSER

Managing Editor of the Journal of Morphology

To serve until December, 1941.....	C. E. MCCLUNG
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Members of Editorial Board of the Journal of Morphology

To serve until December, 1939		
LEIGH HOADLEY	W. A. KEPNER	J. PERCY MOORE
To serve until December, 1940		
J. H. BODINE	N. E. MCINDOO	H. H. PLOUGH
To serve until December, 1941		
J. E. KINDRED	H. W. RAND	S. A. MATTHEWS

PROCEEDINGS OF THE THIRTY-SIXTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOOLOGISTS

RICHMOND, VIRGINIA, DECEMBER 28, 29, 30, 1938

PROGRAM

The annual meeting of the Society was held in conjunction with Section F of the American Association for the Advancement of Science and in association with several other biological societies. With the exception of the joint symposium with the Genetics Society of America, which was held in the auditorium of the John Marshall High School, all sessions for the reading of papers were held in the Academy of Medicine of Richmond. The demonstration program took place in laboratories of McGuire Hall of the Medical College of Virginia.

In addition to the titles and abstracts printed in the December supplement to *The Anatomical Record*, the Society by vote at the annual business meeting authorized the publication of the following late titles and abstracts:

241. THE ESTROGEN-PROGESTERONE INDUCTION OF SEXUAL RECEPTIVITY IN THE SPAYED FEMALE RAT. John L. Boling, Richard J. Blandau and William C. Young, Brown University.

Of twenty-two animals injected with 10 R.U. estrogen (Progynon B) heat (willingness to mate) occurred in six following latent periods from 48 to 54 hours. When fifteen of the remaining sixteen were injected with 0.4 I.U. progesterone (Proluton) on the seventy-second hour, heat occurred in all after latent periods from 1.5 to 4 hours.

Of ten animals injected with 20 R.U. estrogen, heat occurred in two about the fiftieth hour. When the remaining eight were injected with 0.4 I.U. progesterone on the seventy-second hour, heat occurred in all after 2 hours.

Of twenty animals injected with 100 R.U. estrogen, heat occurred in eighteen after latent periods from 38 to 67 hours. In the remaining two injected with 0.4 I.U. progesterone on the ninety-sixth hour, heat occurred after latent periods of about 2.5 hours.

Eighteen of the above animals in which an estrogen induced heat occurred and seven in which an estrogen-progesterone induced heat occurred were subsequently injected with 0.4 I.U. progesterone. A second heat followed in all in the former group, but in only two in the latter group.

These data indicate that in spayed rats as in the guinea pig estrogen induces heat in some animals, but estrogen supplemented by progesterone is more effective. They suggest as a corollary that the mechanism of heat production in rats is similar to that in guinea pigs. The chief differences seem to be that rats are more sensitive to estrogen alone, but when progesterone is required, the effective quantity is larger.

242. SEXUAL DIFFERENTIATION IN *VORTICELLA MICROSTOMA*. Harold E. Finley, Morehouse College. (Introduced by D. H. Wenrich.)

1. This investigation was undertaken to ascertain the origin of conjugating individuals of *Vorticella microstoma*, namely the macroconjugants and microconjugants, and to determine whether there was evidence of sexual differentiation in this ciliate.

2. The macro- and the microconjugant originates from a vegetative (neuter?) individual as a result of an equal division of the micronucleus accompanied by an unequal division of the macronucleus and cytoplasmic components. This division is designated as the preconjugation division.

3 a. The products of a preconjugation division are markedly different in size. Microconjugants are much the smaller of the two and lead a free-living existence. In this investigation they were never known to produce microconjugants nor to encyst. Microconjugants either conjugated with macroconjugants or died of exhaustion and starvation. Food vacuoles were never found within the microconjugant cells even though each microconjugant has a vestibule.

b. In all visible respects macroconjugants closely resemble ordinary vegetative individuals; they undergo vegetative division and also form protection cysts but they cannot *directly* give rise to macroconjugants; if they fail to conjugate, a small number of vegetative generations ensue (ranging from three to ten generations in this stock) and culminate once more in a preconjugation division.

c. Microconjugants never conjugate with ordinary vegetative individuals.

4. The data presented are submitted as evidence of sexual differentiation. The preconjugation division is believed to be the sex differentiation division.

The total number of papers scheduled to be presented in person at Richmond was 135. This is the largest number of 'papers to be read' which has ever appeared on a program of the American Society of Zoologists. According to reports from the presiding officers of the various sessions, 7 papers were omitted. In all, therefore, 128 papers were actually read at the sessions of the Society in Richmond. Twenty-four demonstrations were presented, and 100 papers were included in the program as read by title. (Included in the foregoing figures are papers in symposia, as well as the two late titles and abstracts recorded above.)

All scientific sessions of the Society were well attended. It is estimated that nearly 1000 persons were present at the joint symposium with the Genetics Society on Wednesday afternoon.

The Biologists' Smoker was held on the evening of the first day of the meeting, December 28th, in the auditorium of the John Marshall Hotel, with an estimated attendance of about 1000.

The Annual Dinner of the American Society of Zoologists was held on Thursday evening, December 29th, at the Hotel Richmond with 230 persons present. An address on "Human Psychology and Some Things that Fishes Do" was given by Dr. F. B. Sumner, vice-president of Section F of the American Association for the Advancement of Science.

BUSINESS MEETING

The thirty-sixth annual business meeting opened at 12.10 P.M., December 29th, President M. H. Jacobs presiding.

The following business was transacted:

1. The minutes of the last meeting, having been published in the January 1938, supplement to The Anatomical Record and distributed to all members of the Society, were approved as printed.

2. *Report of the Secretary.*

a. By vote of the Society the Secretary was authorized to publish the two foregoing late titles and abstracts as a part of the Proceedings of the Society.

b. Membership. For the information of the Society the Secretary reported the following changes in membership which have occurred during the last year. (Included in the following figures are new members, which were elected later in the meeting).

Deaths	2	Reinstated	1
Resignations	7	New active members	22
Dropped	5	New associate members	60
Total enrollment at present		896	
Total enrollment in last report		827	
Gain in enrollment		69	

Summary of enrollment

Life members	5
Active members	659
Associate members	232
	—
Grand total	896

3. The Society has suffered the loss by death of two active members, Dr. Maurice Crowther Hall and Dr. George Frederick Laidlaw. The following resolutions were adopted by a rising vote, and the Secretary was instructed to send copies to the nearest relatives and to publish the resolutions in the Proceedings of the Society.

MAURICE CROWTHER HALL

Dr. Maurice C. Hall, chief of the Division of Zoology, National Institute of Health, U. S. Public Health Service, died in Walter Reed General Hospital, Washington, D. C., on May 1, 1938, following an operation for gastric ulcer. Doctor Hall was born at Golden, Colorado, on July 15, 1881; graduated from Colorado College in 1905; and took his master's degree at the University of Nebraska in 1906. In 1907 he came to Washington to begin his work in the Bureau of Animal Industry and in 1925 was appointed chief of the Zoological Division of that Bureau, a position which he held until 1936, when he entered the Public Health Service. During the interval between 1917 and 1919, Doctor Hall was connected with Parke, Davis & Company and served as First Lieutenant in the U. S. Army. During the years of his residence in Washington, Doctor Hall took advantage of educational facilities to obtain his Doctor of Philosophy degree from George Washington University in 1915 and the degree of Doctor of Veterinary Medicine from the same institution in 1916. In 1925, his alma mater, Colorado College, bestowed on him the honorary degree of Doctor of Science.

Doctor Hall's interest in gregarines in student days soon gave way to an enthusiasm for the study of helminths. His early work on the gid tapeworm and related cestodes of carnivores led to the publication of several papers which were followed later by a monograph which is still a standard reference work on the subject. In 1916 appeared his monograph on nematodes of rodents, a work which contained descriptions, illustrations and keys to the genera and species of nematodes from the orders Rodentia, Lagomorpha, and Hydracoidea, and represented one of the earlier attempts at a sound classification of nematodes. An important compilation was the paper on arthropods as intermediate hosts of helminths which appeared in 1929. This paper has been of inestimable value to workers engaged in life history studies.

Doctor Hall is probably best known for his work on anthelmintics. He introduced the method of critical testing of these drugs on experimental animals and laid down sound principles of anthelmintic medication which before had been based mostly on empirical observations or on earthworm testing *in vitro*. With

such a system, he brought order out of chaos in this important field of therapy, established beyond doubt the efficacy of certain known anthelmintics and showed the worthlessness of other drugs popularly employed in medical and veterinary practice. His discovery in 1921 of the value of carbon tetrachloride as an anthelmintic for the removal of hookworms provided the first effective treatment for hookworm disease and led to the widespread adoption of this drug in human and veterinary medicine. In 1925, with Shillinger, he published results of experimental work with tetrachlorethylene for the removal of hookworms, a drug which, because of its greater safety, is largely replacing carbon tetrachloride for this purpose.

In the National Institute of Health, Doctor Hall reorganized the work in parasitology and medical zoology and chose as important research problems trichinosis, oxyuriasis and amoebiasis. He was particularly active in surveying the status of trichinosis in this country, in pointing out the widespread distribution of the parasite, and in urging the adoption of suitable control measures.

Doctor Hall was long a member of the American Society of Zoologists, as well as many other national and international scientific organizations.

W. H. WRIGHT.

GEORGE FREDERICK LAIDLAW

George Frederick Laidlaw was born June 26, 1871, in Jersey City. He graduated from the New York Homoeopathic College in 1890 and practiced medicine in New York until 1926 when he retired. He then studied histological methods with, among others, Nageotte, Rio Hortega, Pierre Masson, and finally Penfield in New York. While in Penfield's laboratory, he developed a special silver technique for reticulin and collagen fibers. When Penfield went to Montreal in 1928, Laidlaw became associated with the Surgical Pathology Laboratory of Columbia University where he remained until his death. He applied his silver technique to demonstrate the endoneurial sheath of reticulin fibers about the cerebral and spinal nerves which begins at the exact point of their emergence from the brain and cord (Plenk-Laidlaw sheath). Laidlaw then began to study melanin, working out a simplified technique for the dopa reaction and studying the distribution of melanin in health and disease. The work culminated in his hypothesis that the human pigmented hairy mole is a link or transition from the pigmented tactile organs of the reptilian type to the hairy tactile organs of the mammalian type. His last work was concerned with the granules in the islet cells of the pancreas. He tried many modifications but never succeeded in obtaining a reliable and satisfactory method for demonstrating them. He died June 22, 1937. George Laidlaw was remarkable because all his productive scientific work was done in the last 11 of his 66 years of life, after 34 years of active medical practice. Into those few final years he crowded achievements which are of greater importance than it is given most men to accomplish in a lifetime.

ARTHUR PURDY STOUT.

4. *Report of the Treasurer.*

The Treasurer presented the following report:

Report of the Treasurer for the year 1938

Credits:

Balance on hand December 28, 1937, when account was last audited	\$1635.04
* Receipts from dues	3020.15
Interest on savings account	25.30

\$4680.49

Expenditures:

Biologists' smoker, Indianapolis	15.00	
Miscellaneous expenses, Indianapolis	62.07	
Third International Congress of Microbiology	50.00	\$127.07
Secretary's expenses:		
Stenographer	46.75	
Printing and stationary	89.84	
Postage and express	15.05	
Traveling	80.95	
Stipend	200.00	432.59
Treasurer's expenses:		
Secretary	60.51	
Postage	39.50	
Printing	7.23	
Miscellaneous expenses	2.95	110.19
Wistar Institute:		
Abstracts	518.34	
Proceedings and list of members	190.44	708.78
Biological Abstracts		1432.00
Bank charges		11.96
* Checks lost by New York Clearing House		12.00

2834.59

Balance December 24, 1938:

Checking account	820.60	
Savings account	1025.30	1845.90

\$4680.49 \$4680.49

Increase in funds since December 28, 1937, amounts to \$210.86

* Duplicate checks for those lost by the New York Clearing House were obtained and credited in the above \$3020.15.

5. *Report of the Auditing Committee* (Thurlow C. Nelson and William H. Cole).

For the Auditing Committee Dr. T. C. Nelson reported that all books and accounts of the Treasurer had been examined, found to be correct and in accordance with the Treasurer's Report as submitted to the Society.

It was voted to accept and approve the reports of the Treasurer and of the Auditing Committee.

6. *Report of the Executive Committee.*

a. *Biological Abstracts.* The Committee recommended the support of Biological Abstracts for 1939 on the same plan as for 1938, namely, that \$2.00 from each membership fee of \$4.00 be paid to Biological Abstracts.

It was voted to approve this recommendation.

b. *Honorary Membership.* At the 1936 annual meeting of the Society a motion was passed directing the Executive Committee to take steps toward making provision for honorary membership in the Society. Such a provision would require an amendment to the Constitution. Therefore, acting on the above motion and in accordance with the stipulations in the Constitution regarding amendments (Article VI), the Executive Committee submitted for consideration of the Society the following proposed amendment to Article II, Section 1 of the Constitution.

Honorary membership: Distinguished biologists, who have established and maintained a high record for outstanding productive scholarship, may be elected to Honorary Membership. Nominations must be made to the Secretary in writing at least three months previous to an annual meeting and must be signed by at least five active members. Each nomination must be approved by unanimous vote of the Executive Committee before presentation at an annual meeting of the Society. Honorary members shall enjoy all the privileges of the Society, but shall be exempt from all dues and assessments.

After considerable discussion of the proposed amendment the following motion was made and seconded: that the proposed amendment be referred back to the Executive Committee for review and reconsideration in the light of the present discussion. The motion was passed by a vote of 35 to 23.

c. *New Members.* In accordance with the stipulations in the Constitution regarding membership, the Executive Committee recommended the following candidates for election to membership in the Society:

ACTIVE MEMBERS

(Formerly Associate Members)

Bailey, Percy Laurence, Jr.	Lynch, James E.	Price, John Worthington
Boughton, Donald Clarke	MacLennan, Ronald F.	Sandow, Alexander
Dempsey, Edward W.	Margolis, Otto S.	Schechter, Victor
Eggleton, Frank E.	Mazia, Daniel	Torrey, Theodore W.
Illick, John Theron	Milne, Lorus Johnson	Turner, C. Donnell
Janes, Ralph Gordon	Nigrelli, Ross Franco	Turner, John P.
Kleinholz, Lewis Hermann	Nuttycombe, John W.	Walzl, Edward McColgan

ACTIVE MEMBERS

(Not formerly Associate Members)

Boell, Edgar John	Hungate, Robert Edward	Osborn, Clinton Morris
Brues, Charles Thomas	Kidder, George Wallace	Pfeiffer, Carroll A.
Dorris, Frances	Lerner, I. Michael	Romanoff, Alexis L.
Figge, Frank Henry John	Lutz, Brenton Reid	Stewart, Colin Campbell
Hamre, Christopher John	Maloeuf, Noble S. R.	Taylor, George Wellford
Hiestand, William Andrew	Myers, Earl Hamlet	van der Schalie, Henry
Hill, Samuel E.	Oliver, Clarence Paul	Walls, Gordon Lynn
Hoagland, Hudson		

ASSOCIATE MEMBERS

Barry, Alexander	Libby, Raymond Loring	Rabinowitz, Morris
Black, Edgar Clark	Lipsett, James Conley	Rahn, Hermann
Bookhout, Cazlyn Green	Lowrance, Edward Walton	Reinhard, Edward G.
Briggs, Robert William	Lund, Everett Eugene	Renshaw, Birdsey
Chase, Hyman Yates	McCutcheon, Frederick H.	Robeson, John Maxwell
Crosman, Arthur Marston	Magruder, Samuel R.	Roosen-Runge, Edward
Deason, Hilary John	Merriman, Daniel	Rosenberg, Lauren Emery
Diller, William Frey	Mickey, George H.	Schell, Margaret Wood
Dugal, Louis Paul	Miller, James Albert	Scott, Florence Marie
DuShane, Graham Phillips	Moreland, George Edward	Sonnenblick, Benjamin P.
Evans, Gertrude	Moser, Floyd	Spotkov, Elias Morlenn
Fry, Frederick E. J.	Nabrit, Samuel Milton	Stanley, Allan John
Hagquist, Carl Waldemar	Noble, Elmer R.	Stiles, Karl Amos
Hammond, Datus Miller	Nunnemacher, Rudolph F.	Tartar, Vance
Hunter, Francis Robert	Odiorne, Joseph Milton	Thomas, Thurlo Bates
Jones, Edmund Ruffin, Jr.	Olson, Magnus	Thornton, Charles Stead
Kimball, Richard F.	Pfeiffer, Isabelle Weed	Todd, Robert Emerson, Jr.
Kitchin, Irwin Clark	Phelps, Lillian Aline	Tobin, Charles Emil
Lawson, Chester Alvin	Piatt, Jean	Wilder, Magel Craig
Levenstein, Irving Max	Porter, Keith Roberts	Whittinghill, Maurice

It was voted that all candidates be elected as recommended by the Executive Committee and the Secretary was instructed to cast one ballot in their favor.

7. Report of the Nominating Committee and Election of Officers.

The Nominating Committee, consisting of G. H. Parker, chairman, W. C. Curtis and Fernandus Payne, presented the following report.

President, 1 year: J. T. Patterson.

Vice-president, 1 year: H. B. Goodrich.

Treasurer, 3 years: T. C. Nelson.

Executive committeeman 5 years: M. H. Jacobs.

Society representatives on Council of A. A. A. S., 1 year: B. H. Willier; C. R. Moore.

Society representatives on Council of Union of American Biological Societies, 1 year: H. W. Stunkard; L. H. Snyder.

Society representatives on Advisory Editorial Board of Biological Abstracts: H. S. Pratt; G. K. Noble; D. H. Tennent; L. V. Heilbrunn.

Representative on the executive committee of the Commission for the Standardization of Biological Stains: S. I. Kornhauser.

Members of editorial board of the Journal of Morphology, 3 years: J. E. Kindred; H. W. Rand; S. A. Matthews.

It was voted that all the above candidates for office be elected as recommended by the Nominating Committee and the Secretary was instructed to cast one ballot in their favor.

8. Report of the Representative on the Executive Committee of the Commission for the Standardization of Biological Stains.

The report of Dr. S. I. Kornhauser, which was received too late to be presented at the business meeting, is as follows:

As representative of the American Society of Zoologists on the Commission for the Standardization of Biological Stains, I wish to report the following activities for the past year:

1. Biological testing of the following dyes: hematein, Nile blue, Sudan III and IV and Congo red.

2. Abstracting methods of technique for Stain Technology.

3. Reading and editing papers submitted to Stain Technology.

4. Giving counsel in devising a method whereby formulae for certified solutions of stains could be included in the National Formulary so that druggists could be guided in making suitable solutions bearing the certification label.

S. I. KORNHAUSER.

9. The president appointed the following Nominating Committee to prepare a slate of officers for 1940: D. H. Tennent, chairman; D. M. Whitaker; Sewall Wright.

10. It was voted to express the sincere thanks of the Society to the members of the Local Committee for the arrangements and the cordial hospitality, which have served to make the Richmond meeting so successful.

11. A motion was made and unanimously passed expressing the thanks of the Society to the retiring Treasurer, Dr. Walter N. Hess, for his efficient services during the last 3 years.

The meeting was adjourned.

E. G. BUTLER,
Secretary.

LIFE AND ACTIVE MEMBERS

(* indicates life members of the Society; † indicates charter members of the American Morphological Society; mail addresses are in italics.)

- ABRAMOWITZ, ALEXANDER A., B.A. (St. Stephen's), M.A., Ph.D. (Harvard), Research Assistant, *Biological Laboratories, Harvard University, Cambridge, Mass.*
- ACKERT, JAMES EDWARD, A.B., A.M., Ph.D. (Illinois), Professor of Zoölogy, Parasitologist, Agr. Exp. Station; Dean, Division of Graduate Study; *Kansas State College, Manhattan, Kan.*
- ADAMS, A. ELIZABETH, B.A. (Mt. Holyoke), M.A. (Columbia), Ph.D. (Yale), Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*
- ADAMS, LEVERETT ALLEN, A.B., A.M. (Kansas), Ph.D. (Columbia), Assistant Professor of Zoölogy, University of Illinois, *406 Nevada St., Urbana, Illinois.*
- ADAMSTONE, FRANK BOLTON, B.A., M.A., Ph.D. (Toronto), Assistant Professor of Zoology, *347 Natural History Building, University of Illinois, Urbana, Ill.*
- ADELMANN, HOWARD BERNHARDT, A.B., A.M., Ph.D. (Cornell), Professor of Histology and Embryology, *Stimson Hall, Cornell University, Ithaca, N. Y.*
- ADOLPH, EDWARD FREDERICK, A.B., Ph.D. (Harvard), Associate Professor of Physiology, School of Medicine, *University of Rochester, Rochester, N. Y.*
- AGERSBOG, H(ELMER) P(ARELI VON WOLD) K(JERSCHOW), B.S., M.S. (Washington), M. A. (Columbia), Ph.D. (Illinois), Consulting Biologist, *U. S. National Park Service, Box 378, Hilliard Road, Dumbarton, Va.*
- ALEXANDER, (EDWARD) GORDON, A.B. (Central), A.M., Ph.D. (Princeton), Associate Professor of Biology, *University of Colorado, Boulder, Colo.*
- ALLEE, WARDER CLYDE, S.B. (Earlham), S.M., Ph.D. (Chicago), Professor of Zoölogy, *University of Chicago, Chicago, Ill.*
- ALLEN, BENNET, Ph.B. (De Pauw), Ph.D. (Chicago), Professor of Zoölogy, University of California at Los Angeles, *909 Micheltorena St., Los Angeles, Calif.*
- ALLEN, EDGAR, A.M., Ph.D., Sc.D. (Brown), Professor of Anatomy, Yale University, *333 Cedar St., New Haven, Conn.*
- ALLEN, EZRA, A.B., A.M. (Bucknell), Ph.D. (Pennsylvania), Associate Professor of Histology and Embryology, *New York Homeopathic Medical College and Flower Hospital, York Ave. and 64th St., New York, N. Y.*
- ALLEN, WILLIAM RAY, A.B., A.M., Ph.D. (Indiana), Professor of Zoölogy, University of Kentucky, *417 Clifton Ave., Lexington, Ky.*
- ALTENBURG, EDGAR, A.M., Ph.D. (Columbia), Assistant Professor of Biology, *Rice Institute, Houston, Texas.*
- ANDERSON, BERTIL G., A.B. (Augustana), M.S., Ph.D. (Iowa), Assistant Professor of Biology, *Biological Laboratory, Western Reserve University, Cleveland, Ohio.*
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FIFTY-FIFTH ANNUAL SESSION

Harvard Medical School, Boston, Mass.

Invitation of Boston University, Harvard University,
Tufts College

April 6-8, 1939

ABSTRACTS

PAPERS

(The numbers indicate the order in which the papers appear on the program of the meeting.)

65. *Studies on intercellular substance in the living rabbit.* Richard G. Abell and Eliot R. Clark, Department of Anatomy, University of Pennsylvania.

These studies represent an attempt to increase our knowledge of the intercellular substance, in two directions:

1. In order to study the pH of intercellular substance in the living animal, a 0.4% solution of phenol red was sealed in the moat. It gradually diffused into the tissue substance, producing a color of sufficient density to permit direct reading of the approximate pH. The color was maintained as long as the indicator remained in the moat (several weeks), without any obviously toxic effect on the living cells. Studies indicate a pH of approximately 7.2 for the intercellular substance of the rabbit's ear tissue during normal circulation. When

* Deceased.

the circulation is cut off, the pH falls to at least 6.8 (the limit of the indicator), as it also does when CO₂ is passed through the moat.

2. Studies of E. R. and E. L. Clark have suggested that the intercellular substance is of a gelatinous rather than a free-fluid nature. This is supported by the observation in the moat chamber that when mammalian Ringer's solution is sealed within the reservoir, it gradually becomes more and more viscous, as substances diffuse into it from the tissue. In an attempt to secure information concerning the nature of this material, many analyses of the total nitrogen of the moat contents have been made, and, while incomplete, they indicate a gradual accumulation of nitrogen containing substances.

36. *Melanin pigment in the central nervous system of vertebrates.* Alexandra Adler, Department of Neurology, Harvard Medical School. (Introduced by George B. Wislocki.)

Great amounts of melanin can be found in the ganglion cells of the central nervous systems of tailless (frogs and toads) and tailed (*Amblystoma*) amphibians during and shortly after metamorphosis. Amphibians which do not metamorphose have either no black pigment (*Cryptobranchus*, *Amphiuma*, *Megalobatrachus*) or (*Siren*, *Proteus*) smaller amounts of it than amphibians with complete metamorphosis.

Some *Plethodontidea* (*Typhlotriton*, *Eurycea*) and *Caecilians* have no melanin pigment in the nerve cells regardless of whether they metamorphose or undergo a direct development.

These findings show that melanin is not an essential ingredient for metamorphosis. In all available series of amphibians in which ganglion cells containing melanin are present, the amount of melanin increases till the height of metamorphosis is reached and then diminishes gradually or disappears completely. It can therefore be considered as a by-product or waste product respectively of metabolism and differentiation of cells during incomplete or complete metamorphosis.

The gradual increase of melanin in the substantia nigra of adult man, which is absent in the corresponding center of infants and of animals, shows certain similarities with the process observed in amphibians. In this connection attention is called to the fact that the substantia nigra is a part of the extrapyramidal system which has a changing functional value in adult man as compared to children and animals.

61. *Differentiation 'in vitro' of the scleral cartilage of the chick embryo.* Rodolfo Amprino, Department of Zoology, The University of Chicago. (Introduced by Paul Weiss.)

The mesenchymal precursor of the scleral cartilage of chick embryos of various ages was explanted 'in vitro' and tested, a) for its capacity for differentiation and regeneration; b) for its structural reaction to mechanical stress (stretching, folding).

Scleral mesenchyme explanted on or after the fourth day of incubation (i.e., 2 days before the normal term of chondrification) produced cartilage in all cases.

This cartilage has the same morphological character, and follows the same course of development, as the normal scleral cartilage of the embryo. It always has the shape of a plate.

Folding of the explant does not produce fusion along the area of contact. The structure of the folded cartilage is modified according to the mechanical stresses. Regeneration of cut cartilage was never observed, even fragments in close apposition after 'intraperichondral' fracture failed to fuse. Explants developing under stretch produced thinner cartilage than unstretched controls; the amount of thinning seemed to correspond to the intensity of stretch.

For comparison, mechanical effects were also studied in living embryos. Experimental collapse of the eye, reducing the scleral tension, resulted in the differentiation of scleral cartilage adapted in shape and size to the diminutive bulb. Such cartilage is thicker than normal; its thickness varies in different points.

37. *Neuronophagia in the cerebral cortex in man and in the mouse as a result of the normal aging process.* Warren Andrew, Department of Anatomy, University of Georgia School of Medicine.

A study of a large number of human brains from specimens ranging from youth through senility has shown a definite process of neuronophagia in senile individuals. A quantitative study of the degree of satellitosis of the glial cells about the large pyramidal cells of the cerebral cortex has been made and an increase with advancing age observed, by far the larger number of satellite cells being found in the individuals over 60 years of age.

Actual ingestion of the nerve cells by the glial cells can be seen in many fields in these individuals. The process is essentially similar to that seen in senile mice, as determined by earlier work and by more recent confirmatory work. It is apparently a cytolysis of the nerve cell body accompanied or followed by ingestion of its substance by the glial cell. The nucleus is more resistant to the cytolytic process than is the rest of the cell.

Neuronophagia of the large pyramidal cells in senile individuals in man is found in both the frontal and occipital cortex, but no increase in degree of satellitosis nor neuronophagia is seen in the Purkinje cells of the cerebellum. Changes in senility in the nerve cells include increase in the basophilic properties of the nucleus, loss of Nissl material, and in some cases the development of pigmentation in the cytoplasm. (See demonstration no. D2.)

18. *The genesis of the ventral longitudinal path.* A. W. Angulo y Gonzalez, The Wistar Institute of Anatomy and Biology, Philadelphia.

The genesis of the ventral longitudinal path is described with especial reference to the formation of the fasciculus longitudinalis medialis. This latter path takes its origin from three chief centers situated in the mid-brain: inferiomedial, interstitialis, and posterior commissural nuclei. Thus the fasciculus longitudinalis medialis is a complex system, and the designation of the nucleus interstitialis as the nucleus of the fasciculus longitudinalis medialis is unwarranted.

The ventral longitudinal path develops progressively in a cephalocaudal direction with the increase in age of the fetuses. In its downward course it sends off

collaterals that, in decussating ventrad of the floor plate, come into synaptic relationship with the motor centers of the opposite side. The ventral commissure also develops progressively in a cephalocaudal direction, appearing successively at the level of the various motor centers. Thus, a nerve impulse that finds its way to this path travels caudad and in so doing, by virtue of its innumerable collaterals, sets the whole motor mechanism into a total pattern reaction, and since these collaterals are crossed the resultant movement has to be, of necessity, a contralateral reaction. Furthermore, since this mechanism is perfected previous to the appearance of the sensory system, it is concluded that the motor system is completely integrated without the intervention of the sensory system.

92. *The renal and suprarenal blood vessels.* Barry J. Anson, James W. Pick and Lindsay E. Beaton, Department of Anatomy, Northwestern University Medical School.

The renal and suprarenal arteries and veins were studied in 200 consecutive cadavers; concerning these blood vessels the following facts are salient.

Supernumerary renal arteries occur on approximately one-third of the 400 sides; they are derived from aorta, superior mesenteric, phrenic, renal, suprarenal, perinephric and spermatic arteries; they are most frequent at hilus, least so at inferior extremity.

Additional renal veins occur in but 14% of the cases; rarely do they leave the parenchyma. Their relation to other vessels is often highly variable; through hiatuses within them pass renal and spermatic arteries; dividing, they encircle the aorta; occasionally the internal spermatic artery hooks around a lumbar communication. Connections with other veins are frequent; renal veins communicate with lumbar, spermatic, iliac, azygos, intercostal veins and with retroaortic plexuses.

Suprarenal arteries are always numerous; they are derived not only from aorta, inferior phrenic and renal arteries, but from internal spermatic, coeliac, superior mesenteric, perinephric and ureteric sources; thirty is the maximum number observed in a single specimen. They are variously related to other vessels, sometimes passing anterior to the renal pedicle, or crossing to the opposite gland.

Unlike the arterial, the suprarenal venous plan is remarkably simple: doubling of the suprarenal vein occurs once; communication with other veins is rare.

88. *Induction of deciduomata in rats by means of progesterone.* E. B. Astwood, Biological Laboratories, Harvard University. (Introduced by F. L. Hisaw.)

The injection of progesterone in castrated adult rats, primed with estrogen, was not consistently effective in sensitizing the endometrium to trauma. Progesterone was fully capable, however, of promoting the growth of deciduomata in normally sensitized uteri. Pseudopregnancy was induced in adult animals by electrical stimulation of the cervix uteri during estrus; this procedure was followed by pseudopregnancy in sixty-five consecutive control animals. Four days after cervical stimulation the ovaries were removed and the endometrium traumatized. In twenty-two control animals whose ovaries remained intact, deciduomata developed in every instance, while in sixteen other uninjected controls, oophorecto-

mized at the time of trauma, no deciduomata were formed. Progesterone injections in the test animals were given over the 3 days following operation, and the resulting gross uterine swelling graded one plus to four plus by comparing with deciduomata formed in 1 to 3 days in normal pseudopregnant rats. A total dose of 1.5 mg. or more gave three to four plus responses; 0.75 mg., two plus; 0.3 to 0.6 mg., negative to one plus. Simultaneous administration of 0.3 γ or more of estradiol inhibited the reaction; smaller doses were without effect. Variation of the volume of the injected oil or subdivision of the daily dose did not influence the response. A rat unit of progesterone is suggested; this is the amount of hormone which when given during 3 days to animals prepared in this way, will result in a two plus decidual response. This unit is equivalent to 0.75 mg. of progesterone.

56. *Differential stain for the nuclei of the suprarenal cortical cells.* Dan D. Baker, Department of Anatomy, Louisiana State University Medical Center, New Orleans.

The nuclei of the cells of the suprarenal cortex of the mouse, rat, cat, bat, dog, guinea pig, rabbit, cattle and man have been differentiated after applying Mallory's triple connective tissue stains to frozen sections. The nuclei of the cortical cells have been divided into four types: dark blue, light blue, red, and yellow. These nuclei were seen in the cortical cells of all three zones. Cells with blue nuclei were the most numerous and those with yellow nuclei the least. In the human suprarenal cortex there was some differentiation of cytoplasm also. Cytoplasm of the cells was yellow, greenish-gray, blue-greenish-gray, and blue.

The cytoplasm of degenerating cells in the cortex was yellow and the nuclei were yellow, red, and blue. (See demonstration no. D3.)

32. *The morphology of the synapse in the cat's spinal cord.* Murray L. Barr, Department of Anatomy, University of Western Ontario. (Introduced by C. C. Macklin.)

End-bulbs of Held-Auerbach, stained by modification 4 of Cajal's reduced silver nitrate method, were studied in spinal cords of thirty mature cats. The average number of endings per unit area of cell surface is approximately the same on ventral horn cells and on cells of the chief portion of the dorsal horn. End-bulbs occur with the same frequency in different segments of the spinal cord. In the best preparations the density of end-bulbs on the surface of ventral horn cells is approximately sixteen per 100 sq. μ . There are probably several thousand endings on the cell body and dendrites of the larger neurones of the ventral horn. About 38% of the cell membrane is devoted to the formation of synaptic junctions with other neurones.

Single end-bulbs vary in size from less than 1.0 μ in diameter to 4.0 μ by 5.0 μ . The smaller end-bulbs predominate on cells of the lateral groups of the ventral horn. End-bulbs of intermediate size predominate on cells of the medial groups of the ventral horn and the chief sensory nucleus of the dorsal horn. The largest endings are infrequent and, when present, occur with those of intermediate size. (See demonstration no. D5.)

19. *Functional development of the fetal brain.* Donald H. Barron, Department of Anatomy, Cambridge University.

During the first half of gestation the movements of the sheep fetus change from 'jerky,' to sustained, and finally toward the seventieth day the limbs often become rigid in extension. From about the eightieth day onward nearly all activity of the fetus is suppressed until birth. These changes in the character of the fetal movements appear to be due to the inhibition of bulbo-spinal mechanisms by the functional development of neurones lying further and further forward in the brain stem; for surgical removal of the appropriate forward parts of the neuraxis causes the older types of movements to reappear again. Results similar to those brought about by surgical procedures can also be produced by asphyxiation of the fetus.

114. *Bio-electric correlates of the menstrual cycle.* Dorothy S. Barton, Neuro-anatomy, Yale University School of Medicine. (Introduced by H. S. Burr.)

A bio-electric study of 156 menstrual cycles in thirty-six women is reported. Over 100,000 determinations were made and analyzed statistically. Daily fluctuations were grouped together in a central tendency and all values outside the central tendency were considered significant peaks. Bio-electric records in rabbits and man supported the assumption that the significant peaks were correlated with ovulation. In the analysis of the menstrual cycles, all were reduced to decils in order that they might be of a comparable length. The significant peaks from the entire group occurred in all decils but there was a distinct grouping of peaks at the mid-interval. This implied that no part of the menstrual cycle was lacking in bio-electric correlates of ovulation and, that ovulation, while occurring more frequently in the middle of the cycle, may occur in any part of the cycle, including the menses. As the number of cycles studied in each individual increased, there was a tendency for a greater spread of significant peaks throughout the period with little or no sign of a rhythm. Records from one menopause, a sterility case, two males, and one operative case showed no such significant peaks.

55. *The blood vessels of the adrenal medulla and their relation to the chromaffin cells.* H. Stanley Bennett, Department of Anatomy, Harvard Medical School. (Introduced by George B. Wislocki.)

Injected and sectioned adrenal glands of cats show that the medulla receives blood from two sources: 1) from radial cortical capillaries which, on reaching the medulla, immediately gather into sinus-like venous radicals which branch extensively and penetrate the entire medulla, closely approximating all chromaffin cells; and 2) from numerous radial penetrating medullary arteries which pass through the cortex without branching and break up in the medulla into an extensive bed of five arterial capillaries. These penetrate all parts of the medulla between the venous radicals, into which they ultimately drain. The arrangement of the arterial capillaries and venous sinuses of the medulla is such that the chromaffin cells are characteristically supplied with arterial capillary blood at one end of the cell, whereas the other end of the cell borders on a venous branch. The Bodian silver technique shows many chromaffin cells to contain numerous

fine black granules, sometimes packing the cytoplasm densely and uniformly, sometimes sparse or lacking, sometimes concentrated at the venous end of the cell. These staining differences suggest different secretory phases of these cells. Furthermore, the pronounced segregation of black silver granules at the venous pole of some cells suggests that the medullary cells discharge their secretion from the venous end of the cell, whereas they probably receive nutriment and oxygen chiefly from the arterial capillaries at the opposite pole.

40. The development of the limbs in urodeles as influenced by prolonged refrigeration. Isabel Harper Blount, Department of Anatomy, University of Minnesota.
(Introduced by R. F. Blount.)

An attempt to analyze the reasons for the abnormality of the limbs in urodele larvae kept continuously in the refrigerator brings out the fact that cold itself is not the immediate cause, nor has darkness anything to do with it. The limbs of animals kept at 6 to 9°C. develop normally at first, but later shrink in many of their dimensions until sometimes only stumps without digits remain. This occurs whether the animals are fed or not. However, since a similar process sometimes takes place in animals starved at room temperature the resorption would appear to be due to a lack of normal nutrition in the refrigerated animals through poor circulation. As has been shown by Coghill ('36) the gills, tails and other structures are also affected. The resorption in animals starved at room temperature is not always pronounced, and may escape observation, since death occurs so soon. In the refrigerator metabolism is so much lowered that the animals will live about 7 months without food. Histologically the reduced limbs show a progressive disappearance of cartilage and muscle tissue beginning distally. The muscles of the body in general in refrigerated animals whether fed or not and in those starved at room temperature show great wasting and few fibrillae.

20. The question of developmental dependence for the hypophysis upon the brain. Raymond F. Blount, Department of Anatomy, University of Minnesota.

Earlier we found no growth of the hypophysis as a transplant to a heterotopic position in the embryo of *Ambystoma*. Similar results were obtained by Atwell although his grafts were successful in the frog. In 1935 our results showed that following brain removal (Harrison's stage 31) without injury to the hypophyseal rudiment in *Ambystoma* no development of the pars intermedia occurred but that a poorly developed anterior lobe was present. The individuals lacked normal pigmentation and showed vasodilation. Those results have now been extended showing that the thyrotropic hormone is produced by the underdeveloped anterior lobe although no animals have metamorphosed. The age at which the brain may be removed and allow some anterior lobe development has been followed back to stage 24 which is before any thickening for the rudiment has occurred. Further extension of the series is in progress. The pars intermedia does seem dependent upon the pituitary floor of the diencephalon for its development. If only a few cells of the brain are deliberately left in the transplant they give rise to a vesicle containing practically nothing but the thinned floor to which the pars intermedia

is applied. These experiments bring the results upon the frog and those upon the urodele more nearly into agreement, although why heterotopic transplants of the one develop and of the other do not must be further studied.

64. *Mitotic rhythm in the epidermis of the albino rat.* Charles M. Blumenfeld, Department of Anatomy, University of Utah.

To determine the presence of a rhythm in mitotic activity 28-day-old male albino rats were killed at various intervals of the day and night. Skin was removed from the center of the ventral abdominal wall, fixed in Bouin's, cross sectioned at 8μ , mounted serially and stained with Harris' hematoxylin and eosin Y. The number of mitoses observed in 1000 consecutive fields was taken as an index of mitotic activity in a specimen. Mitoses in the epidermis alone were included. The first 44 of 240 specimens have been studied. These were from rats killed as follows: 10 between 8 A.M. and 2 P.M., 14 between 2 P.M. and 8 P.M., 10 between 8 P.M. and 2 A.M. and 10 between 2 A.M. and 8 A.M. For each time interval the mean number of mitoses per 1000 fields was, respectively, 70, 30, 16 and 33. A comparison with mitotic activity in the renal cortex of the same rats, previously communicated, shows that both epidermis and renal cortex exhibited least mitotic activity during the first half of the night, that greatest activity occurred during the first half of the day in skin and during the second half of the day in renal cortex.

31. *Further cytological observations on the vertebrate synapse, with special reference to axonal endings in certain tegmental centers of the goldfish.* David Bodian, National Research Council Fellow in Medicine, Marine Biological Laboratory, Woods Hole, Mass.

In perfused Zenker-formol material, stained with Mallory-azan, many structural characteristics of the synapse and its environment are demonstrable, which are inadequately shown by the silver impregnation methods. A probable uniformity of structure and size range occurs in most vertebrates, but the largest endings are difficult to preserve. The extremes of form and size in the favorable goldfish material are represented by the smallest endfeet (less than 0.5μ), and the largest club endings (about 7μ in diameter at the terminal surface). Transitional endings occur, and both endfoot and club types may occur on terminal branches of a single fiber. This is found in the complex endings on the cells of the oculomotor nuclei, and probably also in similar basket-like endings. All endings appear to have an accumulation of mitochondria near the terminal membrane, which is adherent to the cell of termination. The size range is only partly dependent on the caliber of the parent fiber, but the smallest endfeet are generally terminals of the finest fibers, which are usually unmyelinated. The small endfeet may occur on the proximal part of the axon, as well as on perikaryon and dendrites. The distribution of the small endfeet on certain cells is extremely regular, with inter-distances about equal to the diameter of the endfeet. The distribution of the Purkinje cell dendritic thorns is similar, and these thorns have many of the other characteristics of the smallest endfeet.

6. *Respiratory metabolism of mammalian eggs and embryos.* Edgar J. Boell and John S. Nicholas, Osborn Zoological Laboratory, Yale University.

The respiratory metabolism of rat eggs and embryos during the early stages of development has been measured by means of the Cartesian diver ultramicromanometer in correlating certain aspects of physiological activity with particular morphological states. The delicacy of the method can be gauged by the fact that the oxygen consumption of as few as six to ten eggs, corresponding to a dry weight of approximately 0.2γ , can be measured with a fair degree of accuracy.

The eggs were removed by excising the tubes at definite intervals after copulation. These were cut into small pieces which by peristaltic activity extrude the ova. The embryos were removed by surgical exposure of the uterus and delivery of the embryos within their deciduomata. The decidua was dissected and the embryo freed from decidua and superficial membranes. The preparation of both eggs and embryos was performed as rapidly as possible.

The oxygen consumption averages 0.00073 c.u.mm. per egg per hour. No significant difference has so far appeared in the amount of respiration performed by one-cell and eight-cell eggs. The respiration of isolated embryos at stage 12 (8 day) is approximately 0.01 c.u.mm. per embryo per hour. During the next 2 days of development there is a twentyfold increase in the amount of oxygen consumed. The increase in oxygen utilization parallels the increase in embryonic mass.

120. *The maternal ovaries and sexual behavior of the young and the mother at parturition in the guinea pig.* John L. Boling, James G. Wilson and William R. Fish, Department of Biology, Brown University. (Introduced by William C. Young.)

Data bearing on the heat response shown by male and female guinea pigs at birth and on the postparturitional heat of the mother are being studied with respect to the occurrence or absence of postparturitional ovulation in the mother.

Of 82 young females and 99 young males in 66 litters, all but one female and two males gave the heat response. Data bearing on the postparturitional heat exist for sixty-two of the mothers. Heat periods whose length averaged only 3 hours, range 0.5 to 10 hours, occurred in thirty-nine. In the seven animals of this type whose ovaries were examined before February 24th ovulation had occurred. In twenty-three mothers postparturitional heat did not occur although the young gave the heat response. In the seven animals of this type whose ovaries have thus far been examined, ovulation had occurred in four, but not in three.

It is apparent, therefore, 1) that the heat response in young at birth is almost invariable, 2) that its occurrence is not dependent on either heat or ovulation in the parent, and 3) that postparturitional heat in mothers is less frequent and shorter than heat in unmated animals. The possibility that the postparturitional heat responses of the young and the mothers are stimulated by different mechanisms is being investigated and will be discussed.

67. *On the size of the cells in the striate and parastriate area of some primates.* Gerhardt von Bonin, Department of Anatomy, University of Illinois.

The nuclear size of the nerve cells in the striate area (OC) and in the margo magnocellularis of the parastriate area (OB γ) has been studied and correlated with the distance of the individual cells from the surface. The brains of man, orang and cebus were used.

In the fifth and sixth layers the correlation between depth and size is expressible by a straight line. In all three primates, nuclear size increases in those layers with depth. The gradient is of practically the same steepness in both OC and OB γ . It is steepest in man, less so in orang and flattest in cebus.

The average size of the nuclei is largest in man, and least in cebus. Both the solitary pyramidal cells in OC, V (of Meynert) and the large pyramidal cells in OB γ , III show the same trend, but the nuclei of the giant stellate cells of OC, IV B are of the same size in all three brains.

47. *The effect upon the red cells of newborn rats of administering liver and gastric preparation to the mother.* Estelle Briese and Geo. M. Higgins, The Mayo Foundation.

Normal human gastric juice, when administered to pregnant rats, has been found effective in reducing the mean cell diameter and corpuscular volumes of the erythrocytes of the blood of the newborn. Whether this is a maturation effect or the result of stimulation cannot be stated. Schlicke ('39) has shown that gastric juice, of patients with pernicious anemia, when given to pregnant rats, is without effect upon size or volume of the erythrocytes of the newborn.

This report covers observations which have been made upon the number, size and volume of erythrocytes of newborn rats, born to 1) a group of mothers which received extralin by mouth, 2) a group of mothers which received reticulogen intramuscularly and 3) to a group which ate whole liver as a major portion of their diet. Hog, dog, horse and calf liver was tested.

Results indicate that either liver concentrates or whole liver is less effective than gastric juice or the stomach preparations, when given to pregnant rats, in reducing the mean red cell diameter of newborn.

124. *Prolonged vaginal bleeding, fetal resorption, and prolonged gestation in the Albany strain of rats.* Ethel Burack, J. M. Wolfe and A. W. Wright, Departments of Anatomy and Pathology, Albany Medical College, Union University, Albany, New York.

The Albany strain of rats, in addition to an unusually high incidence of spontaneous mammary fibroadenomata in the females, is characterized by low fertility. One factor which contributes to this is the occurrence of spontaneous fetal resorption and prolonged gestation. In this study 400 pregnancies in the Albany strain and 133 control pregnancies in rats of the Vanderbilt strain, in which tumor incidence is low and fertility high, were analyzed. Vaginal smears were made daily. The time of onset of macroscopic vaginal bleeding (placental sign), the number of days during which bleeding continued, and the length of each gestation were noted. Uterine contents were palpated daily from the first day

of bleeding. In the Albany strain the average time of onset of vaginal bleeding was 12.7 days. Bleeding persisted for an average of 5.2 days. The mean gestation period was 22.5 days, but 9% of deliveries occurred on the twenty-fourth day or later. The average number of young was 5.4 per litter. In 16% of pregnancies complete fetal resorption occurred, and 25% of the animals resorbed at least once. In the controls the average first day of bleeding was 13.5; bleeding persisted for an average of 2.9 days. The mean gestation period was 21.8 days; the average number per litter was 8.7. Fetal resorption occurred only once.

100. *The intrinsic muscles of the avian hand.* Berry Campbell, Department of Anatomy, University of Oklahoma School of Medicine.

The homologies of these muscles in the bird with those of mammals and reptiles is particularly hard because of alteration of the extremity, loss of parts, and shifting of innervation. Available literature is based entirely on topographic and functional grounds. Concomitant study of chick embryos and adults of various species has clarified many of the problems. There is a dorsal short extensor layer which differentiates into an extensor pollicis brevis, an extensor indicis brevis, the dorsal interosseous, and a dorsal abductor metacarpi tertii. The deepest ventral layer forms the flexor pollicis brevis, the volar interosseous, and a ventral abductor digiti tertii. An intermediate layer, deep to the long flexors, gives rise to a flexor pollicis intermedius and a flexor digiti secundi brevis. Ontogeny does not reveal whether the abductor of the thumb is an extensor or a flexor element.

When these conditions are compared with those found in the turtle, the loss of muscles is conspicuous. Absent are not only those for the missing digits, but also the intermetacarpal, the flexor brevis minimus, the flexor brevis superficialis, and the interphalangeal groups. The flexor brevis profundus series is represented by but one layer in each metacarpal space.

111. *Thermal and capillary effects on the quantitative variability and orientation of muscle cross striae.* Eben J. Carey, Department of Anatomy, Marquette University School of Medicine.

Sudden temperature elevation, 37°C., 10 seconds, then depression 0°C. of muscle, skinned frog in Locke's solution, cause local contraction waves, many with bi-concave ends by capillarity. Cross striae multiply two to four times in nodes. Higher temperature causes heat rigor; striae multiply four to ten times with turbulent internal structure. Gradual temperature elevation, 37°C., causes generalized striae multiplication throughout fiber, two to four times that, 17°C. Z-bands are not constantly present. Frogs and bees maintained, 8°C., 1 to 14 days, have progressive granulation and subtraction of oriented cross striae in many muscle fibers.

Pressure nodes of striped fiber contraction waves have differential chemical staining, wide diameter, condensed cytoplasm and rounded nuclei, multiplication fine striae, mineral ash and anisotropy increase. Tension internodes have narrow diameter, rarefied fibrillated cytoplasm, greatly elongated nuclei, coarse striae, ash and anisotropy decrease.

Travelling overlapping peristaltic waves transform into standing striped pressure system by ligatures or transverse intestinal incisions, 1 inch apart, followed by stimulations. Gizzard muscle contraction waves multiply by limited temperature changes.

Changeable muscle striae and contraction waves are variable expressions of chemical compression waves, of differential energetic levels, of periodic reversible, or irreversible exposable molecular reactions of metabolism. Relatively constant volume plus limited temperature changes, equal, increased internal muscle compression during isotonic and isometric tensions. (See demonstration no. D11.)

43. *An experimental study of the relation of sensory control to motor function in amphibian limbs.* Philena Chase, Department of Anatomy, Columbia University. (Introduced by Samuel R. Detwiler.)

Complete or partial deafferentation of the brachial nerves of *Amblystoma* embryos was accomplished by several methods. In the majority of cases, a close correlation was found between the extent of development of normal motor function and the amount of afferent innervation. In only a small number of cases did typically normal motor patterns develop in limbs with very deficient afferent innervation. In a few instances, limbs with some sensory fibers lacked function. Despite these few exceptions, the bulk of evidence favors the view that afferent innervation plays a significant role in the building up of brachial motor patterns.

Deafferentation of the limbs of the adult *Triturus* by ganglionectomy did not immediately effect the coordination and perfection of their use. After 6 weeks, it was noticed that the deafferented limbs showed some hyperextension. This condition became progressively more severe until it was customary for the affected legs to rest in an extremely hyperextended state, with severely dislocated joints. This observation is particularly interesting, in view of those of Weiss ('35) who found that the function of the limbs of adult toads and axolotls was not limited by deafferentation. Although the deafferented limbs typically showed no 'spontaneous' movements during general activity of the animal, the capacity for normal motor function was apparently not lost, since normal coordinated movements occurred upon excessive sensory stimulation.

87. *Observations on the nuclei of stromal cells in human endometrium.* Rucker Cleveland, Department of Anatomy, Vanderbilt University School of Medicine.

Following Bouin fixation and hematoxylin-eosin stain, two arrangements of chromatin, either granular or solidly homogeneous, were noted in a study of the functional stroma of biopsy samples of human endometrium. In the granular arrangement, the granules were arranged in two sharply contrasting modes, termed aggregate, or coarse, and diffuse, or fine. In the first, they were aggregated along lines approximating those of the linin network, the intervening nucleoplasm being relatively free of chromatin particles; in the second, they were diffusely distributed throughout the nucleoplasm, with one or more large condensations of chromatin. The nuclei of specimens in which mitotic figures occurred usually had the aggregate type of granular chromatin. The solidly homogeneous nuclei in the functional stroma resembled the nuclei characteristic of the basal is.

They were more often found in the stroma of specimens in which the granular chromatin was of the diffuse type of distribution. Nuclear characteristics of human endometrium in various phases of the menstrual cycle were compared. Aggregate or coarse chromatin predominated in the proliferative phase. Tissues of the secretory and menstrual phases of the cycle presented a much more varied picture of granular chromatin. (See demonstration no. D12.)

99. *Constant occurrence in adult bodies of a connective tissue layer which is the fascia of fusion of Toldt expanded to keep pace with the growth of the body and not greatly modified from its original form.* Edgar D. Congdon, Department of Anatomy, Long Island College of Medicine.

Fifty unilateral series of frozen sections of the abdomen in well-hardened bodies when traced and dissected revealed a constant occurrence of a frontally placed fascia extending on the left side from the anterior surface of the suprarenal or left diaphragmatic crus caudally as far as the attachment of the pelvic colon to the pelvic brim. Almost constantly its lateral border attaches to the peritoneum where this sheet bends anteriorly from the parietes to the wall of the omental bursa and attaches at a corresponding location in the paracolic gutter. Medially the fascia ends somewhat variably, and if it reaches the aorta, overlaps its anteriorly. It always continues with the posterior fascia of the pancreas. Part of it constitutes the anterior layer of the renal fascia.

A similar layer on the right side usually extends cranially only a short distance beyond the attachment of the transverse mesocolon.

The fascia on the two sides constitutes a topographical boundary plane in the abdominal cavity second in extent and significant relations only to the peritoneal cavity itself. Enteric organs and blood vessels always lie anterior to it, as also do the spleen and its vessels. Suprarenal, kidney and internal spermatic vessels lie posterior to it, and the ureter also except for a distance where the medial border of the fascia may withdraw lateral to this tube. (See demonstration no. D14.)

54. *Hyperplasia and necrosis of lymphatic tissue after diphtheria toxin.* Eleanor A. Conway, Department of Anatomy, The University of Chicago.

Lymphatic tissue of guinea pigs reacts first (4 to 7½ hours) to minimal lethal doses of diphtheria toxin by lymphocytopoiesis in preexisting nodules and formation of many new ones. There is also a marked increase in movement of lymphocytes from the lymph nodes and their accumulation in the lungs. Death of lymphocytes first appears 12 hours after the injection of the toxin and progresses in amount so that the centers of the nodules become necrotic. Antitoxin given within the first 6 hours prevents necrosis of lymphocytes; if given within 12 hours it stops further necrosis of them; if given within 24 hours lymphocytopoiesis begins in necrotic centers. Preliminary experiments indicate that the necrosis of lymphocytes is in part, but not entirely, dependent on necrosis in the adrenal cortex, and that if cortin is administered with the antitoxin and for several days thereafter both lymphatic tissue and adrenal cortex will regenerate, even if antitoxin is given after necrosis of lymphatic tissue has occurred. The

end, or necropsy, picture of the lethal action of diphtheria toxin shows many reaction centers in the nodules; these are secondary changes in lymphocytopoietic nodules. Guinea pigs fed on a diet insufficient in vitamin C are more susceptible to diphtheria toxin than those on an adequate diet.

76. *The sensory innervation of the spinal accessory and tongue musculature in the rhesus monkey.* K. B. Corbin and F. Harrison, Department of Anatomy, Stanford University; Institute of Neurology, Northwestern University School of Medicine; and Division of Anatomy, University of Tennessee College of Medicine.

Using cathode ray oscillograph and speaker, action potentials characteristic of proprioceptive impulses, were elicited by stretching the spinal accessory muscles, and were followed centrally over the spinal accessory nerve and its anastomotic branches with the upper (second, third and fourth) cervical ventral rami. These experiments were checked by means of degeneration studies.

Muscle sense fibers supplying the sternomastoid and cleidomastoid muscles in the monkey leave the accessory nerve to course centrally in C2 and C3 ventral rami. Fibers of similar function from the superior and inferior trapezius muscles leave the accessory to run in C3 and C4 ventral rami. Faradic stimulation of these rami and their anastomotic branches with the accessory never caused contraction of the accessory muscles.

These results, and previous work on the cat and rabbit, indicate that the accessory musculature is supplied with proprioceptive fibers whose cells of origin are located in the upper cervical dorsal root ganglia. These fibers pass peripherally over the respective cervical ventral rami, usually anastomosing with the spinal accessory nerve before entering the accessory muscles. In none of our experiments could evidence be found that such fibers accompany the accessory nerve through the jugular foramen.

Degeneration studies of the hypoglossal nerve following cervical ventral rami section revealed a small contribution of sensory (probably proprioceptive) fibers from C2 to the tongue musculature.

78. *Further observations on the ratio of nerve cells to fibers in the dorsal root.* H. A. Davenport and Fred C. Holmes, Department of Anatomy, Northwestern University Medical School.

A new staining method for spinal nerves (Davenport, McArthur and Bruesch, '39) has made feasible a restudy of the cell to fibers ratio in dorsal roots and their associated ganglia. The number of nerve fibers in all the roots from segments C1 to S3 in the cat, together with the number of cells in the ganglion belonging to each root, was determined. A total of thirty-six roots were studied and of these nineteen showed agreement between the number of cells and number of fibers to within 5% or less (number of cells = 100%). Seven gave cell excesses of 6 to 10%, and seven of 11 to 14%. One, a C2, gave a cell excess of 28%, while one S2 gave a fiber excess of 10% and one T9 gave a fiber excess of 38%. In general, the conclusion of Duncan and Keyser ('38) that a 1:1 ratio of cells to fibers in dorsal roots of cat is the rule has been confirmed, particularly in the lower thoracic and upper lumbar region where our data agree

with theirs. The large cell excesses seen in C2 may be due to imperfection in fixation of these specimens. The cause of a rather consistent excess of cells over fibers in the upper thoracic nerves leaves the ratio in this region open to question. The large fiber excess in the one T9 indicates that this was an atypical nerve while the 10% excess of S2 is within outer limits of error.

102. An anomalous pericardial sac and liver in a cat exhibiting an almost complete absence of the diaphragm. C. D. Day, Department of Zoology, Westminster College.

An anomalous pericardial sac and liver found in a cat which exhibited an almost total absence of the diaphragm are described. Embryological interpretations are offered. It would seem from a study of this specimen that the septum transversum is not necessary for the completion of the pericardial sac; furthermore, that the liver can develop in the total absence of the septum transversum, and that when it does its only anchorage is the ventral body wall. Its axes are determined by the ventral mesentery of the foregut into which it grows and from which it obtains its entire serosa.

12. The relationship between the central nervous system and the reproductive cycle in the female guinea pig. Edward W. Dempsey, Departments of Anatomy and Physiology, Harvard Medical School.

Ovulation was observed in adult female guinea pigs after various surgical lesions in the central nervous system, including destruction of the superior and inferior colliculi, the pretectal area, the septal and anterior preoptic areas, and the posterior part of the mammillary bodies. Likewise, ovulation occurred in six of ten animals in which the pituitary stalk had been sectioned.

The evading responses in dioestrous guinea pigs and the oestrous responses of animals in heat, both of which are evoked by stroking the hair on the back or rump, were also studied. Destruction of the tectum, while not interfering with ovulation, abolishes these responses. Undercutting one superior colliculus was followed by a loss of response to contralateral stimulation only. Removal of the pretectal area, or a midline section between the corpora quadrigemina, were without effect.

These data constitute evidence that the previously described afferent tract involved in sexual behavior runs forward through the tectum and turns ventrally at the level of the superior colliculus. Ovulation in the guinea pig, and the control of the sexual cycle, however, appear to be independent of this tract, and of any nervous pathway reaching the hypophysis through the pituitary stalk.

46. Experimental production of a leukemoid state. Tom Dougherty, Department of Pathology, University of Oklahoma School of Medicine. (Introduced by Berry Campbell.)

Guinea pigs were used as the experimental animals. Active agent was lymph from the thoracic duct of a dog. Animals were kept under constant temperature and free from infection both previous to the injection, and during the experimental phase. Control counts, total counts and differentials were made previous to the experiment.

Following the injection, the total count remains rather constant with an immediate lymphopenia which passes into a lymphocytosis in the later stages. At the third week the most characteristic findings are anemia of a macrocytic type (index 1.2), a low hemoglobin (approximately 37), a lowered total leukocyte count and a proliferation of young cells of a reticulo-endothelial nature. A large number of normoblasts in all stages of development, transitional forms between the reticulum cells and lymphocytes, also cells of the reticulum and myelocytes are found in peripheral blood. The most significant histological changes are found in the spleen, the liver, the lymph nodes, lung, kidney and suprarenal glands. There is an extensive erythropoiesis in the spleen and the medulla of the suprarenal gland. There is also an extensive production of monocyte like cells in the spleen. The lymph nodes contain many macrophages, blood pigments and extravasated red blood cells; there is a general hyperplasia. The lung, kidney and liver present a picture similar to that of leukemia. (See demonstration no. D17.)

52. Regeneration of lymphocytes and bone marrow cells in normal laboratory animals. Hal Downey and Dorothy Sundberg, Department of Anatomy, University of Minnesota.

Renewed study with the imprint method and May-Grünwald-Giemsa staining confirms conclusions expressed in previous papers. The myeloblast of the bone marrow is not identical with any of the most immature lymphocytes of lymph nodes of adult animals but does resemble some cells of the nodes of newborn rabbits. In the adult the cytoplasm of the myeloblast is less basophilic and the chromatin pattern of the nucleus shows finer texture due to its extremely thin chromatin strands which form a delicate network. There is little or no clumping of chromatin, and nucleoli, when present, have sharper outlines than in immature lymphocytes. As myeloblasts are scarce in the adult most of the myeloid regeneration is homoplastic.

Heteroplastic development of lymphocytes from reticulum is a continuous process and results in the presence of cells which grade from one type to the other. These cells are usually large and very basophilic, and most of the mitotic figures of our material were in these cells. However, mitoses of mature lymphocytes of all types prove that they are not a stem cell for lymphocytes in the sense that the myeloblast is for myeloid cells. The nuclear pattern of the intermediate lymphocytes is composed of sharply outlined chromatin granules or rings embedded in acidophilic parachromatin and shows gradually increasing clumping of chromatin as the cells develop to lymphocytes.

62. On the structure and the function of the ellipsoids of the spleen. P. Dustin, Jr., Harvard University and University of Brussels, Belgium. (Introduced by Frederic T. Lewis.)

The 'Capillärhülsen' of Schweigger-Seidel, or 'ellipsoids,' are thick cellular sheaths surrounding the splenic capillaries. Similar structures have been described in vertebrates of most zoological groups. Several theories have been proposed to explain their function; valvular action, regulation of the blood flow,

sphincters (Knisely), growth centers of splenic reticulum, phagocytosis, endocrine activity. No theory, however, can explain their fundamental function, unless applicable to all species. The principal facts shown by comparative and experimental histology are: 1) the ellipsoids and the endothelium of the capillary they surround are permeable to blood, i.e., the spleen circulation is 'open'; many of the blood cells and most of the plasma flow through the ellipsoids themselves; 2) the cells of the ellipsoids have a quite different physiology than any other splenic cell. They play a very small part in iron or pigment storage, but important accumulations of fat can be found in these structures in fishes, birds and mammals; 3) in some fishes, a great number of destroyed red blood cells can be found in the Schweigger-Seidel sheaths; 4) the size of the sheaths varies considerably in most animals for unknown reasons.

The function of the ellipsoids cannot be defined; it may, however, be related to fat metabolism and hemolysis. Further research may thus bring a better understanding of the functions of the spleen. (See demonstration no. D19.)

42. *The morphogenesis of a hind limb of a 60-hour chick transplanted to a turkey embryo.* Herbert L. Eastlick, Department of Zoology, University of Missouri. (Introduced by L. J. Wells.)

The right posterior limb bud of a 60-hour White Leghorn embryo was transplanted to the lateral body wall of a turkey host of the same age. The host, which was normal in appearance but retarded in size, died on the nineteenth day of incubation. The graft became well incorporated with the tissues of the host and developed into a normal appearing leg in which all skeletal elements are present.

The musculature appears to be normal when examined macroscopically. Moreover, cytological study reveals that considerable areas of the graft are composed of typical striated muscle fibers. Other regions, however, have undergone extreme fatty degeneration. In these areas a heavy infiltration of lymphocytes has occurred.

Although the limb was obtained from a White Leghorn donor, the graft possesses pigmented feathers. The feathers adjacent to the point of attachment are as darkly pigmented as those of the host while the down distal to the point of attachment is not fully colored. It is postulated that melanophores of the host have migrated into the transplant and have fed melanin into the developing follicle cells of the graft.

97. *The action of two-joint muscles.* Herbert Elftman, Department of Zoology, Columbia University.

The sequence of action of the two-joint muscles of the leg in walking has been recorded by means of action currents. Comparison of this sequence with a record of the muscular forces present shows that during certain portions of the step the two-joint muscles can produce the observed movement while doing less work than would be required if only one-joint muscles were present.

21. *On the nature of the thyrotropic field phenomenon in the tadpole of Rana pipiens and R. sylvatica.* William Etkin, American Museum of Natural History and City College, New York.

It had previously been reported that when the primordium of the thyroid and pituitary are transplanted near each other precocious activation of the thyroid with consequent precocious metamorphosis was induced. Further experiments showed the following.

1. Histological evidence of the activation in the graft is first seen about 7 days after operation, when the normal thyroid first differentiates follicles. The activation appears to reach its peak about 20 days after operation, the gland thereafter usually appearing inactive though large.

2. Small but differentiated thyroids respond as primordia do.

3. A thyroid primordium implanted near the pituitary may later be found, activated, in the orbit. Yet primordia introduced directly into the orbit show no activation. Apparently the field does not extend into the orbit. General developmental considerations make it seem probable that a thyroid that makes its way into the orbit must do so during the first day or two after the operation. If this is so it would appear that the pituitary primordium is surrounded by a thyrotropic field and that the thyroid primordium is sensitive to this.

4. Typical cases of activation were obtained by implants of thyroid primordia into the pituitary region of differentiated tadpoles of 15 mm. total length. The activated glands were found touching or nearly touching the pituitary, non-activated thyroids along the sides of the infundibulum.

22. *Relations between the growth promoting effects of the anterior pituitary and the thyroid hormone.* Herbert M. Evans, Miriam E. Simpson and Richard I. Pencharz, University of California.

Experiments show: 1) growth promotion in excess of normality secured by anterior pituitary extracts is not conditional on the presence of the thyroid, but 2) is greater when the thyroid is present, and 3) in thyroidectomized animals becomes equally great if thyroxin is concordantly administered. Since the restoration of normal growth, produced by thyroxin given to thyroidectomized animals, is interpretable as due to stimulation of the pituitary, thyroidectomized animals were also hypophysectomized, when it was seen that thyroxin no longer influenced their growth rate. Anterior pituitary extract conferred the same growth on hypophysectomized-thyroidectomized animals as upon thyroidectomized ones, and here also the treatment of doubly operated animals with thyroxin and anterior pituitary extract led to the same maximal growth seen in both normal and thyroidectomized animals similarly treated. The experiments with the doubly operated animals show that 1) the stimulation of the growth of thyroidectomized animals can occur only in the presence of the pituitary, and 2) that the synergism between growth promoting extracts and thyroxin is equally demonstrable when the combined treatment is given animals from which both glands have been removed.

83. *Functional synergism of the follicle stimulating and luteinizing hormones of the pituitary.* H. L. Fevold, Department of Biology, Harvard University. (Introduced by F. L. Hisaw.)

The follicle stimulating and the luteinizing hormones of the pituitary have been shown to be complementary to each other in producing hypertrophy of the ovaries of rats and in stimulating secretion of male hormone from the testes. The amount of male hormone which is produced by injections of LH free of FSH is sharply limited in 21-day-old normal male rats or in hypophysectomized rats. When FSH, which alone is incapable of stimulating the interstitial elements to secretory activity, is present, the amount of secretion as judged by seminal vesicle weights is markedly increased. Similarly, the amount of estrogen secretion induced by FSH, as measured by uterine weights, is greatly increased by the simultaneous injections of LH which itself does not produce any secretion even in large doses. In these experiments the gonadotropic hormones were injected twice daily for 2 days and the ovaries and uteri weighed 48 hours after the first injection.

Quantitatively, the uteri are no more sensitive to 'pure' FSH than are the ovaries under these conditions. With unfractionated extracts (those containing FSH and LH) the uteri respond more quickly than do the ovaries but the response is a measure of the combined effects of the two hormones and the results cannot be compared to those obtained from another preparation which may contain different amounts of FSH and LH.

103. *Pigment formation: its control, significance, and relation to redox potentials.*

Frank H. J. Figge, Department of Anatomy, University of Maryland School of Medicine.

The indophenol dyes induce a pallor in amphibian larvae by inhibiting the activity of the enzyme tyrosinase in the melanophore. Coincidentally, these same dyes accelerate the activity of the tyrosinase in the connective tissue cells of the same animal. This differential reaction is explained on the basis of the relative redox potentials of the cells and the experimentally determined fact that the activity of the enzyme tyrosinase is regulated by the redox potential of its environment. The dye, which tends to maintain a relatively high positive potential, thus shifts the potential of both cells in this direction, the melanophore away from, and the connective tissue cell toward the optimum redox potential for melanin production by tyrosinase. The final stage of melanin formation is a process of reversible oxidation, the color changing from light tan to black. We thus have an enzymatic reaction which is regulated by the cytoplasmic redox potential; and melanin (the end-product) is capable of exerting an influence on this. According to this concept, the very presence, color, and relative abundance of melanin in any cell may be used to estimate the oxidation-reduction potential of that cell. Developmental, physiological, or even pathological changes in pigment content of cells may be regarded as a manifestation of a shift in the redox potential of the cell.

104. *Biochemical changes associated with onset of secretory activity in the metanephros of the fetal pig.* Louis B. Flexner, Department of Anatomy, Johns Hopkins University.

The object of this study has been to investigate chemical changes occurring in the developing metanephros when inactive nephrons change to secretory organs. Cytochrome oxidase and redox potentials were investigated. Cytochrome oxidase, estimated with Nadi reagent, was found to have this distribution: in nephrogenic zone (undifferentiated nephrogenic tissue, metanephric vesicles, and the S-shaped stage) it is in low concentration and equally distributed throughout. In active tubules its concentration is high; no difference was noted between proximal and distal convolutions. It is absent or almost so in Bowman's capsule and in stroma, and in low concentration in epithelium of collecting tubules.

Redox potentials were determined with indicators. The potential (E') of structures and undifferentiated tissue of the nephrogenic zone was $+0.034$ volt; of epithelium of active proximal and distal convolutions, $+0.100$ volt; of stroma and epithelium of collecting ducts, -0.130 volt.

Results indicate onset of tubular secretory activity is accompanied by these changes: 1) In pre-secretory stage cytochrome oxidase is in low concentration and equally distributed between epithelium and stroma. In secretory stage, oxidase increases markedly in epithelium and disappears from stroma. 2) In pre-secretory stage, redox potentials of epithelium and stroma are alike. With onset of secretion, the potential of epithelium rises and that of stroma falls so that a difference of 0.230 volt develops between them.

77. *A quantitative study of the axons in normal and degenerated cervical sympathetic trunks.* James O. Foley and Franklin S. DuBois, Department of Anatomy, University of Alabama.

Evidence derived from the enumeration of axons and sheaths, in intact upper cervical sympathetic trunks and in comparable stretches of the trunks of cats in which the preganglionic efferents and possible afferents had been eliminated, indicates that: 1) 20 to 73% of the 5,000 to 10,000 axons found in the trunks are unmyelinated, 2) there is no anatomical basis for support of the view that afferents are present, 3) 15 to 46% of the fibers of the intact nerve remain after either radicotomy or ganglionectomy and it is believed that these are largely postganglionic in origin, 4) approximately 90% of the preganglionic axons to the superior cervical ganglion are contributed by the first four thoracic nerves and, finally 5) some sympathetic trunks may have as few as 5% or as many as 60% of their preganglionic fibers unmyelinated.

4. *The development of the adrenal cortex of Alligator mississippiensis.* Thomas R. Forbes, Department of Anatomy, Johns Hopkins University.

The anlage of the alligator adrenal cortex is an area of coelomic epithelium on the ventro-medial mesonephric surface. The anlage extends medially to the root of the mesentery and is continuous laterally with the germinal epithelium of the gonadal anlage; the medial boundary of the latter is only indicated by the presence or absence of germ cells. Both anlagen become thickened by cellular

proliferation and project cords dorsally. The adrenal cortical cords extend rapidly into the mesenchyme between the mesonephric glomeruli and the aorta, branch and become tortuous, and lose their connection with the epithelial anlage. Before hatching takes place the adrenal medullary (chromaffin) tissue, migrating ventrally, comes to rest as islets of intensely basophilic cells between the pale, intertwined cortical cords. Cortical and medullary tissues are never segregated into peripheral and central zones, respectively, of the adrenal gland. The latter retains its contact with the gonad.

The common epithelial origin and topographic relationship of gonadal medullary and adrenal cortical cords is suggestive in the light of current theories of a possible androgenic activity of the adrenal cortex.

90. *Sex hormones and bone changes in mice.* W. U. Gardner and C. A. Pfeiffer, Department of Anatomy, Yale University School of Medicine.

Observations have been completed on 350 mice which have received weekly doses of estradiol benzoate of 100, 250, 500 and 1000 I.U. or such amounts of estradiol benzoate in conjunction with 0.625, 1.25 or 2.5 mg. of testosterone propionate per week. Animals of both sexes from six different inbred strains (Dr. L. C. Strong's colony) were removed after periods of treatment of 50 to 550 days. The skeletons were examined by x-ray photographs, ground preparations of the larger bones and histologically. Animals were maintained on a diet of Purina Fox Chow.

The marrow of the femurs of mice receiving estrogens for 50 days or more showed invasion of osseous spicules appearing first distally and eventually filling the entire cavity. The spicules enlarged with further treatment until the marrow cavities were largely replaced by compact bone. Males responded more slowly than female. At the same time osseous invasion of the marrow of the femurs, sternums, calvaria and other bones occurred as indicated by x-ray shadows, the pelvis were partially reabsorbed and became separated at the symphyses by interpubic ligaments. Mice receiving similar amounts of estrogen but any one of the amounts of testosterone propionate mentioned above showed no apparent changes in the skeleton. The marrow spaces remained normal and the pubes usually remained firmly united at the symphyses. The age of the animals at the onset of treatment affected the rapidity and extent of the change.

72. *The connections of the nucleus of the basal optic root in various mammals.*

Lois A. Gillilan, Mary E. Woolley Fellow, Mount Holyoke College, Department of Anatomy, University of Michigan. (Introduced by Elizabeth C. Crosby.)

Rodent, insectivor, carnivor, chiroptere, and primate brains were used in this study, experimental and normal material being employed. The nucleus of the basal optic root lies behind the nucleus subthalamicus, between the medial tip of the cerebral peduncle and the posterior third of the mammillary body in the shrew, rodent, and primate material, in the position in which other observers have described it, but is farther caudal in the bat and the carnivor. Its neurons are small to medium sized and are oval or round multipolar in type in the bat and the rat; they are larger and elongated with large, pale nuclei in the cat and the monkey.

The experimental material documents previous accounts that the basal optic root is of retinal origin. This root crosses in the caudal portion of the optic chiasm and, after accompanying the main optic tract for a short distance, turns off and runs caudally and medially along the base of the cerebral peduncle to the nucleus of the root. In the carnivor and primate material the root is either very thinly medullated or unmedullated but is well medullated in the other forms considered and is proportionately large in the shrew. Other connections of the nucleus of the basal optic root include fibers passing dorsally to the region of the oculomotor nucleus, fascicles entering the supramammillary decussation, and intranuclear fibers.

28. The effect of p-hydroxy-prophenyl-benzene (anol) in mammary development.

E. T. Gomez and C. W. Turner, Department of Dairy Husbandry, Missouri Agricultural Experiment Station.

Like naturally occurring estrogens, p-hydroxy-prophenol-benzene (anol) is capable of stimulating the growth of the duct system of the mammary gland of normal and castrate female rabbits, rats and male mice. Unlike estrogen, however, anol also stimulates growth and proliferation of localized to generalized lobule-alveolar units of the mammary gland of these animals. When treatment immediately precedes ovarian hormone stimulation, anol induces rapid hyperplasia of the peripheral ends of the ducts present at the time of the initiation of treatment thus presenting a sharp demarcation between the partially and completely developed area of the gland. In addition, numerous cysts and few small nodular masses were observed in the developed area of the gland. Histologically, these nodules consist mainly of dense fibrous growth and acinar cells.

91. The normal, the acromegalic and the hyperplastic nephritic human nephron: a further consideration of the plastic reconstructions of Louis A. Turley.

Allan L. Grafflin, Department of Anatomy, Harvard Medical School.

Three beautiful wax reconstructions of the human nephron, made over 20 years ago by Dr. Louis A. Turley, will be described. The first model, of the normal nephron, represents the only instance in which the reconstruction of an essentially complete adult mammalian nephron—including the entire loop of Henle—has ever been successfully accomplished. The second model represents the glomerulus and proximal convoluted tubule in a case of acromegaly, and is entirely unique. The proximal convoluted tubule has undergone a striking increase in length, but not in diameter, and the increase in length is roughly proportional to the enlargement of the kidney as a whole (approximately twice normal). The third model represents the hyperplastic nephron in chronic nephritis, and exhibits as its outstanding feature a massive enlargement of the proximal tubule; the pars recta shows a remarkably tortuous course, being folded back and forth upon itself. The distal tubule shows no significant abnormality. The glomerulus is considerably enlarged, but on histological grounds its functional capacity must have been markedly reduced.

Golgi first showed that the ascending limb of the loop of Henle applies itself to the vascular pole of the glomerulus of the same nephron. Contrary to Hamburger and Peter, who emphasized the relationship of the ascending limb to the vas efferens, Turley was the first to appreciate its essential relationship to the vas afferens.

107. *Skeletal differences among inbred strains of mice.* E. L. Green and P. B. Swain, Arnold Biological Laboratory, Brown University. (Introduced by William C. Young.)

Examination of eight strains of inbred mice shows they may be grouped on the basis of similarities in the axial skeleton. Four strains (C57Blk, twenty generations inbred; Leaden, forty; A albino, fifty; N short-ear, five) have twenty-six presacral vertebrae, differentiated as seven cervical, thirteen thoracic, and six lumbar. C57Blk and Leaden which have a common ancestry have further similarities in a split xiphisternum and in a 35% occurrence of cervical ribs (?). Two strains (dba, thirty; C₃H, 40) also related by origin have twenty-five presacral vertebrae, 7/13/5. Two strains (Bagg albino, forty; L, five) lack morphological constancy. In Bagg 75% have twenty-six presacral vertebrae, 15% have twenty-seven, and 10% are asymmetrical. In addition 85 to 90% have extra ribs in varying expression at the thoraco-lumbar border. Sixty per cent have seven sternbrae rather than six. The L has 90% twenty-six presacral vertebrae, 10% twenty-seven, and less than 1% twenty-five. Extra ribs occur at the thoraco-lumbar border in 50% and at the cervico-thoracic border in 10%. This is the least inbred of the eight strains and shows the greatest variability. These variations will be useful for a genetic investigation of the problem of sacralization, for a study of extra-genetic factors causing variability within an inbred strain, and for an examination of the developmental mechanics of the sternum.

80. *Some effects of androgens and gonadotropic preparations on the reproductive system of hypophysectomized adult male rats and rabbits.* Roy. O. Greep, Squibb Institute for Medical Research, New Brunswick, N. J.

Hypophysectomized male rabbits receiving human Cyonin immediately after operation showed a much slower rate of testicular regression than untreated operated controls. Sperm formation was not interrupted, the interstitium and secondary sexual organs were stimulated and mating was observed. When the treatment was delayed for 10 days some animals showed a slight increase in spermatogenesis; testicular regression was usually halted. The major reaction, however, was the expected hyperplasia of the interstitium and repair of the accessories. These results, with both the immediate and delayed treatments, have been essentially duplicated with dehydroandrosterone or testosterone propionate except for a lack of interstitial cell stimulation.

A trypsin digest of pituitary (horse), gonadotropic extract injected into hypophysectomized adult rats (tenth to twentieth postoperative days) brought about a pronounced gametogenic action as evidenced by an abundance of live sperm in the fresh testicular smears. The interstitial cells were not restored and the scrotum, seminal vesicles and prostate glands showed no evidence of androgenic stimulation.

98. *An x-ray study of male pelves.* William Walter Greulich and Herbert Thoms, Departments of Anatomy, Obstetrics and Gynecology, and the Adolescence Study Unit, Yale University School of Medicine.

Recent x-ray studies disclosed that only 5.7% of 104 student nurses had the type of pelvis which is described as 'normal' in textbooks of anatomy and of obstetrics and that only 13.5% had the kind of pelvis which, according to the anthropological literature, is proper for white women. In 73% of them the pelvic index was 95 or more.

In order to determine whether the pelvis of the present-day male differs as much as does that of the female from its textbook counterpart, pelvic roentgenograms were made of seventy medical students. The shape of their pelvic inlet was found to vary widely, but approximately 75% of them had pelvic indexes between 95 and 122 and were, therefore, dolichopellic. These are considerably higher than the indexes of from 77 to 84 which were reported for European males by Turner, Topinard, etc., in the nineteenth century and which are still cited in modern textbooks.

As a group, the medical students of our series, like the student nurses previously studied, appear to have attained something at least approximating their maximum normal growth. It is noteworthy that in both groups this definitely superior physical status is associated with a relative elongation of the true conjugate diameter of the pelvis.

Lantern slides showing antero-posterior and lateral views of representative male pelves accompany the paper.

3. *The developmental plan of the mammalian gonads.* Peter Gruenwald, Cook County Graduate School of Medicine, Chicago. (Introduced by L. B. Arey.)

Gonad development is characterized by the formation of three kinds of structures from the tissue of the genital ridge: one of them, the sex cords, is auxilliary to the sex cells; another one, the rete, originally provides for drainage; and the third one, the interstitial cells, probably has hormonal functions. Much discussion has been raised as to the relation of these three types of structures to each other, and even transitions between them have been found under normal and abnormal conditions. A consideration of the embryological facts shows many common characteristics in the origin of the structures in question. This leads to a conception of gonad development which facilitates the understanding of the mutual relations of the parts of this organ. They all develop as epithelial cords from the tissue of the genital ridge and the differentiation proceeds after the principle of division of labor. Such specialization, however, is not strictly and irrevocably determined. This explains the possibility of transitions and makes a strict separation of the origin of different elements of the gonad unnecessary for many considerations. In all discussions of tumor formation, regeneration, or transformation of the gonad this close relation of its parts should be considered. The sex cells proper probably have an exceptional position outside of this system.

118. *The effect of riboflavin and adrenal cortex extract on ovulation in the bat.*

Mary J. Guthrie, Department of Zoology, University of Missouri.

Female bats of the genus *Myotis* are in a condition of submaximal oestrus from October to April. During this time a single follicle with antrum is present in each individual and grows only slightly until the pre-ovulatory period. It has been possible to stimulate such follicles to complete their growth and differentiation and to rupture by means of Collip's gonadotropic extract of the hypophysis, antuitrin S, and Hisaw's luteinizing hormone. In addition, ovulation has been induced by combined dosage of riboflavin (Merck) and adrenal cortex extract (Upjohn). Experiments indicate that administration of an aqueous suspension of riboflavin by mouth is more effective than subcutaneous injection and that twice-daily treatments are better than daily.

Adrenal cortical hormone has been reported to be necessary in the phosphorylation of riboflavin (vitamin B₂) to form the prosthetic group of the yellow oxidation enzyme of Warburg and Christian. A search for interpretation of hormonal coordinations in terms of cell metabolism is invited by the finding that an increased amount of raw material for the production of an enzyme, the function of which is believed to be that of oxygen carrier to the substrate, will induce ovulation. The number of variables which must be considered and the early stage of this investigation do not warrant speculation as to the initial place of effect of the stimulating compound.

89. *Relation of certain pigmentation changes in the human skin to sex hormone substances.* James B. Hamilton, Department of Anatomy, Yale University School of Medicine.

Changes have been observed in the skin and hair of castrated and eunuchoid men during periods of treatment with male hormone substance, testosterone propionate. Direct observation and spectrophotometric analyses, which were done in cooperation with Dr. Edward Edwards, show an increase in blood flow and pigmentation in the skin.

The evidence suggests that the process of tanning in the human skin is photographic in nature, requiring an 'exposure' as to light or heat and a 'development' which depends on sex hormones or similar substances. Exemplary is a 'castrated' man who, upon exposure to sunlight, tanned very poorly. When he was given androgen treatment several months later, however, the previously exposed areas of the skin became tanned.

As in the men, the skin of bilaterally ovariectomized women may have a pasty sallow color. The skin is more pigmented when the women receive either androgenic or estrogenic substance and later loses this color when treatment is withdrawn. The photographic nature of the tanning process is also seen in women.

In some of the men and women of this group whose head hair was partly gray it was observed that the hair had a darker appearance during treatment and again returned to a lighter color after the cessation of treatment.

5. *Some problems in the study of polyembryony in mammals.* G. W. D. Hamlett, Baltimore, Maryland.

Among all vertebrates, only some armadillos and certain human families regularly reproduce polyembryonically. Attempts to analyze experimentally the physiological mechanism controlling polyembryony have failed in the armadillo, due partly to surgical difficulties and partly to refractoriness of this animal to the usual hormones. Available evidence indicates that delayed implantation, found also in many other mammals, bears no causal relationship to the twinning.

In analyzing the occurrence of polyembryony in man, we must recognize at least three embryological processes involved in human twinning. One in fraternal twinning, where separate embryos develop from separately fertilized ova. This is almost certainly unrelated to polyembryony, although certain biologists have tried to prove statistically an association between the two. Secondly, some families produce identical twins with great frequency, generation after generation. This is comparable to the specific polyembryony of *Dasypus*, although the hereditary factors are seldom sufficiently pure or sufficiently dominant to render every birth multiple. Significantly, offspring in these families are normal, whether single or twins. Finally, we find double monsters and Siamese twins produced sporadically by aberrations in embryology, not by any regularly controlled developmental process. Double monsters do not recur, as do twins, nor do they occur frequently in twinning families; the two phenomena are unrelated. Teratology and experimental production of double monsters may explain human Siamese twins; they can throw no light upon hereditary normal polyembryony.

7. *On a complete normal 12-day human ovum of the pre-villous stage.* Arthur T. Hertwig, and John C. Rock, Free Hospital for Women, Brookline, Massachusetts; Departments of Gynecology, Obstetrics and Pathology, Harvard Medical School, Boston and Department of Embryology, Carnegie Institution of Washington. (Introduced by Dr. George L. Streeter.)

This surgically obtained 12-day ovum was located on the posterior aspect of the fundus uteri. Unfixed, it appeared as a raised, translucent area with a sharply outlined, hemorrhagic, marginal ring. Fixed, the area, 1.09×1.1 mm., projected 0.3 mm. above endometrium which incompletely covered it. It was sectioned in faultless series by Dr. C. H. Heuser, the trophoblastic vesicle yielding $158\ 6\text{-}\mu$ sections. External chorionic dimensions are $0.835 \times 0.540 \times 0.948$ mm. The trophoblastic shell, thickening from 0.0125 mm. at abembryonic pole to 0.185 mm. at embryonic pole, consists of syncytial and cytotrophoblastic elements, the former abutting abruptly against pre-deciduous endometrium. Within syncytium are numerous, irregular, communicating, blood-filled lacunae and lesser numbers of varying sized, non-communicating, fluid-filled vacuoles. Cytotrophoblast is giving rise to mesenchyma and angioblasts. Dimensions of the chorionic cavity are $0.425 \times 0.550 \times 0.498$ mm. Within the latter is a secondary cavity, $0.290 \times 0.410 \times 0.474$ mm., imperfectly lined by a membrane of almost endothelial thinness, although ventral to the embryo its cells are polyhydral, more numerous, closely packed together, and arranged in two or more layers. The embryo, without apparent longitudinal axis, consists of a disk of large, vertically arranged,

columnar cells, measuring $0.204 \times 0.165 \times 0.045$ mm. The amnion now forming, apparently from primitive mesenchymal cells, only incompletely closes the cleft-like amniotic cavity. (See demonstration no. D25.)

8. *The 'Yerkes A' chimpanzee embryo.* Chester H. Heuser, Department of Embryology, Carnegie Institution of Washington.

This specimen, interstitially embedded as in man, had not yet produced an elevation of the uterine mucosa. It is slightly smaller than the 'Miller' ovum and is the youngest stage seen in man or the apes. The endometrium has not completely closed over the egg. The ovum, about 0.72 mm. in diameter, entered the mucosa near a large uterine gland and has partially surrounded branches of the gland. A portion of the latter is detached and lies free within the chorion. The chorionic wall is differentiated into cytotrophoblast and plasmoditrophoblast which show marked cellular and staining differences. Within the plasmoditrophoblast are lacunar spaces which by reconstruction are found communicating with each other to form a labyrinthine arrangement. The chorionic cavity is greatly reduced in size; within it are strands of primitive mesoblast. Reconstructions of the germ disk show that it is oval in outline and measures $0.114 \times 0.110 \times 0.058$ mm. A cephalocaudal axis of the embryo is apparent. The amniotic cavity is a slit-like space measuring $0.96 \times 0.07 \times 0.01$ mm. Closely applied to the ventral surface of the germ disk there is a sheet of primitive entodermal cells—a single layer of cells in the caudal region and thickening at the head end in preparation for the formation of the yolk sac.

48. *Experimental sulphanilamide anemia in white rats.* George M. Higgins and Thomas E. Machella, Division of Experimental Medicine, The Mayo Foundation.

Sulphanilamide (para-aminobenzene-sulfonamide) has been given by mouth to white rats. Amounts given daily to four groups of animals were 0.25 gm., 0.50 gm., and 1.00 gm. and 2.00 gm. per kilogram of body weight. Daily food and water intake were measured. Peripheral blood was examined at intervals and preparations of bone marrow were made at autopsy. Toxic reactions observed include, cyanosis, anorexia, loss of weight, thirst, distended stomachs and convulsions. Data will be presented on the total number of erythrocytes and leucocytes, the volume of packed red cells, the mean corpuscular volume, the diameters of the red cells, hemoglobin percentages and extent of reticulocytosis.

Twelve animals which received 2.0 gm. per kilogram body weight for 10 days became rapidly anemic. Total erythrocytes dropped from 9.782 millions to 5.691 millions. There was an increase in corpuscular volume, in cell diameters and in reticulocyte percentages. Twelve animals which received 1.0 gm. daily sustained similar reactions. Six animals received 0.5 gm. of the drug per kilogram; and six received 0.25 gm. per kilogram.

At the end of 10 days, following administration of sulphanilamide in doses of 2.0, 1.0, 0.5, and 0.25 gm. per kilogram, there occurred a reduction in the total erythrocyte count from normal levels, of 42.2, 36.1, 10.2 and 2.9% respectively.

79. *Male gonads in a genetic female goat.* R. T. Hill, Department of Anatomy, Indiana University School of Medicine, Bloomington.

A goat, born a female, started a very pronounced development of the clitoris at about 6 months of age. No mammary gland tissue could be palpated. At about 8 months of age a mid-ventral laparotomy was performed and a castrate type uterus was found. At the distal end of each uterine horn were gonads which had the gross appearance of testes. One was removed and serial sections showed it to be entirely testis, all tubules of which were sterile, with many containing only Sertoli cells. The testis carried normal rete tubules and efferent ductules which connected to a normal and activated epididymis. No vas deferens was present. The distal end of the uterine horn ended blindly.

About 6 weeks later the second gonad was removed, and was found to be identical with the first. However, a vas deferens was present, running parallel to, and closely applied upon the wall of the uterus. The epididymis connected normally to the vas deferens, but the uterine horn ended blindly.

No ovarian tissue was present in either gonad, and the histological picture of uterine glands suggests that no functional ovarian tissue was present in the organism. The gonads had a positive androgenic function as exhibited by an enlarged clitoris and the presence of active epithelium in the vas deferens and the epididymis.

121. *Vascular reactions of the uterus of the immature rat.* R. B. Holden, Biological Laboratories, Harvard University. (Introduced by F. L. Hisaw.)

The vascular responses of the uterus of the immature rat have been studied by the intravenous injection of colloidal mercuric sulfide and by direct microscopic observation of living uterine vessels transilluminated by means of a 'Lucite' rod. Cleared mounts of injected uteri were prepared at intervals after a single injection of 0.1 γ estradiol. Vasodilatation, most marked in the mesometrium, was noticeable in 1 hour, became maximal at about the sixth hour and then slowly regressed; by 24 hours it had largely disappeared. This response was not influenced by the simultaneous administration of 8 mg. of atropine. Vascular responses to various drugs, locally or systemically applied, were studied by direct observation with transillumination. Locally applied, adrenalin (1:100,000) and pituitrin caused prompt and prolonged blanching, whether the uterine vessels were normal or hyperemic following estradiol or histamine. Dilute solutions of acetyl choline (1:1,000,000) were without effect; the local application of a 1% solution produced marked but brief vasoconstriction of a normal or hyperemic uterus. This effect was inhibited by atropine but was not potentiated by eserine, and eserine alone (1:1000-1:100,000) had no local effect. Estradiol caused no vasodilatation within $\frac{1}{2}$ hour when directly applied. Histamine produced marked vasodilatation and removed the venous stasis produced by vasoconstrictors. The vasodilatation induced by estradiol parallels the early increase in weight and water content; it is probably brought about by a vasodilator substance released from specific uterine cells under the influence of estrogen.

24. *Relation of parathyroidectomy and of season to adrenal cortical lipid in albino rats.* Margaret M. Hoskins and Joseph G. Bernstein, Department of Anatomy, College of Dentistry and the Graduate School, New York University.

By means of weighed paper tracings the distribution of lipid in the adrenal cortices of thirty-seven parathyroidectomized and twelve control rats was studied.

Parathyroidectomy was performed when the animals were 90 days old and autopsies from 1 to 90 days after operation. No effect of parathyroidectomy on the lipid content of the adrenal cortex could be detected, at any period. Attempts to correlate the variation of the extent of the lipid containing zones with the serum-Ca level, sex, diet and weight of the parathyroidectomized animals were also unsuccessful. When the cases, both operated and controls, are grouped according to the season in which the animals were killed, mathematically significant differences in the distribution of the cortical lipid are apparent. The widest extension of the lipid containing zones is found in animals killed in the months of May and June. These zones are narrowest in rats killed in January and December, while those autopsied in February and March are intermediate in this respect.

70. *The human nucleus of olfactorius anterior.* Tryphena Humphrey, Department of Anatomy, University of Pittsburgh.

This account is part of a study of the nucleus olfactorius anterior, the tuberculum olfactorium, and the amygdaloid complex of man undertaken in collaboration with Elizabeth Crosby of the University of Michigan. The preparation of the material (toluidin blue serial sections of the adult brain) was aided by a grant from the Horace H. Rackham School of Graduate Studies at Michigan.

The bulbar portion of the human nucleus olfactorius anterior consists of cell groups showing no significant arrangement. Throughout the crus the pars dorsalis is well represented, but the other portions of the nucleus are indicated only by occasional small clumps of cells. As the crus widens out toward the hemisphere the pars dorsalis becomes continuous with the neopallial cortex. The pars lateralis is connected with the pyriform lobe cortex by groups of neurons along the base of the hemisphere. A poorly developed pars medialis could not be traced into continuity with the anterior continuation of the hippocampus as in the macaque. The pars ventralis is represented by a few neurons ventral to the ventricle in the region in which the tuberculum olfactorium appears farther caudally. There is no definite pars externa. In no region does the anterior olfactory nucleus form a complete ring around the obliterated olfactory ventricle, although this relationship is suggested at the caudal end of the crus.

1. *Transmission of antianemic principle across the placenta and its effect on the primitive erythroblasts of the 11-day rat embryo.* Oliver P. Jones, Department of Anatomy, University of Buffalo Medical School.

Pregnant rats were injected parenterally with massive doses of liver extract (Lederle) on the seventh to tenth days after insemination. Dry smears of 11-day embryonic blood were prepared from the yolk sac according to the method described by Kirschbaum (Toronto meetings). Price-Jones curves were constructed from the average cell and nuclear diameters obtained from camera lucida

tracings at 1000 $\times$ magnification. The decrease of mean nuclear diameter is greater than that of the mean cell diameter and the decrease of the former is roughly proportional to the amount of liver extract administered. The percentage of mitoses was not markedly influenced but polychromatophilia, pyknosis and karyorrhexis were definitely increased. These changes are interpreted to indicate an acceleration of cytoplasmic differentiation and nuclear maturation. Emphasis is placed upon the fact that a superabundance of antianemic principle did not cause the transformation of primitive erythroblasts of the prehepatic period into normoblasts or definitive red blood cells but speeded their maturation toward primitive erythrocytes. This response is similar to the one observed in pernicious anemia patients after liver therapy, in which the megaloblasts of the bone marrow do not transform into normoblasts but complete their maturation as reticulated and mature megalocytes. Sectioned material is being studied to determine if cells of the second generation have appeared precociously and to determine the activity of the endothelium. (See demonstration no. D31.)

119. *Histological observations on the mechanism of ovulation.* Virgene Warbritton Kavanagh, New York Botanical Garden, and Fred F. McKenzie, Animal Husbandry Department, University of Missouri, U.S.D.A., cooperating.

At the beginning of estrus in the sheep, the follicle containing the oocyte which is to be released at ovulation has a moderately low granulosa layer of cells which appear to form a syncytium, a well defined membrana propria, a rich vascular net in the theca interna, and a relatively thick theca externa entirely surrounding the follicle. As the liquor folliculi increases in volume, the theca layers become thinner and the blood vessels more conspicuous. About an hour before ovulation the cells between the oocyte and the membrana propria undergo what may be hydrolysis. On the surface side of the follicle the theca externa is thinned by the swelling follicle until only a few strands of connective tissue remain, and even the blood vessels in the theca interna are compressed. First the cells in the theca interna, and then the cells near the surface in the stratum granulosum, are luteinized, destroying the syncytium. A portion of the liquor folliculi escapes through this area reducing the pressure on the follicle wall as a whole but increasing it on the surface forming the ovulation cone and breaking the connective tissue strands and the flattened surface epithelium. Through this break the liquor folliculi and the oocyte with its mass of cells can escape.

63. *A quantitative study of the histology of the secondary lymphoid nodules of the submaxillary lymph nodes of 20-day-old albino rats.* James E. Kindred, Department of Anatomy, University of Virginia.

Two hundred and seventy-two secondary nodules from fifty-seven submaxillary lymph nodes of eleven 20-day-old albino rats were used. The nodules were grouped by area of mid-vertical section into five classes: 1) 600–2000; 2) 2000–4000; 3) 4000–6000; 4) 6000–12,000; and 5) above 12,000 square micra. Differential counts showed that small lymphocytes decreased in relative number as the nodules increased in size; intermediate small lymphocytes increased with increase in size of nodule; medium-sized, large and giant lymphocytes showed no significant differences in distribution between smallest and largest nodules. Reticulum cells

decreased in percentage from smallest to largest, while reticulum cell macrophages increased in percentage with increase in size of nodule. Mitosis rates: no changes in rate of mitosis of medium-sized lymphocytes from smallest to largest nodules (ca. 4%); significant increase in mitotic rate of large lymphocytes from smallest to largest nodules (9 to 16%); fluctuations without regard to nodule size characterized the mitotic rates of giant lymphocytes (11 to 18%); low rates of mitosis of reticulum cells except in largest nodules (0 to 7%). The mitotic rates for nodules as a whole showed definite tendency to increase with size of nodule. Medium-sized and large lymphocytes form the greatest percentage of all cells in mitosis in the nodules. In contrast, the giant lymphocytes have greatest percentage in cortex; and giant and large lymphocytes in the medulla.

82. *Gonad-hypophyseal relationship in the English sparrow, Passer domesticus L.*
Arthur Kirschbaum, Carroll A. Pfeiffer and Jean Van Heuverswyn, Department of Anatomy, Yale University School of Medicine.

Juvenal male sparrows exposed to added daily light (4 P.M. to 11 P.M.) during October, November and December showed complete testicular development within 6 weeks. Juvenal females showed no response during this period when placed under the same experimental conditions. If experiments were begun in January incomplete ovarian response was evidenced in some cases. Injections of pregnant mare serum produced testicular development in juvenal males, whereas juvenal females kept under the same laboratory conditions did not respond to such injections (June to January). These preliminary experiments, in addition to the fact that males mature earlier than females in the spring, suggested that the testis is more responsive than the ovary to gonadotropic secretion. The following experiment was carried out to test this hypothesis.

Undeveloped juvenal testes were grafted into juvenal females, autoplasmic testis-grafts in castrate males serving as controls. Operated birds were exposed to added daily light beginning December 22nd. In 5 instances testis-grafts in females underwent pronounced hypertrophy within 4 to 6 weeks, whereas the hosts' own ovaries had failed to enlarge. The bills of these animals darkened, indicating secretion of male hormone by the grafts. Microscopically mature spermatozoa were evident in testes grafted into females and males exposed to added light.

It is concluded that the testis was more responsive than the ovary to hypophyseal secretion, and that both male and female hypophyses were stimulated by light.

49. *Effect of cobalt on erythropoiesis in normal and anemic rabbits.* William Kleinberg, Albert S. Gordon and Harry A. Charipper, Department of Biology, Washington Square College, New York University.

Cobalt has been shown to exert a marked erythropoietic effect in normal animals. The present experiments were extended to include the influence of cobalt on rabbits rendered anemic either by bleeding or by benzol treatment.

Adult rabbits made anemic by repeated bleedings and maintained on identical diets attain normal erythrocyte levels more quickly and show greater numbers of reticulocytes if 10 mg. cobalt is injected per day.

Animals in another series were injected daily with gradually increasing amounts of benzol for 5 weeks. Those rabbits which showed approximately equal intensity of anemia were selected and divided into two groups. One group received injections of 10 mg. cobalt along with the benzol whereas the other group was injected with the same amount of benzol alone. In the cobalt-benzol animals there occurred a sharp increase in reticulocytes accompanied by a gradually increasing red cell count. Comparisons of femoral bone marrow taken from the same region revealed aplasia in the benzol animals but marked regeneration in the cobalt-benzol rabbits.

Examination of the bone marrows of the cobalt treated normal and anemic rabbits shows nest-like arrangements of erythrocytic cells which appear to be derived from megakaryocyte-like cells in a manner comparable to that described by Jordan in the yolk sac of the pig and in typhoid bone marrow.

53. The lymphocyte in acute inflammation. Fred Kolouch, Jr., Department of Anatomy, University of Minnesota. (Introduced by Hal Downey.)

Tissue spreads, fixed by drying in air and stained with blood stains (May-Grunwald Giemsa) gives superior preparations for the study of the cellular exudate associated with acute inflammation. The sacrifice of tissue architecture necessitated by this technique is more than offset by improved cellular detail and material that allows comparison with blood smears.

In young adult rabbits, in which cutaneous irritation was avoided by mechanical depilation, areas of inflammation were produced by the subdermal injection of 0.4 cc. of irritants such as egg albumen. Tissue for biopsy was obtained from these areas at short time intervals during the first 24 hours and then at daily intervals until healing was completed.

In a study of the lymphocyte in acute inflammation, observation of this process from the eighth to the fourteen hour after onset is essential. At this time only, the transformation of lymphocytes to macrophages can be easily traced. Studies made on inflammations of 18 hours or longer duration yield little information on cellular origins.

The first response of the rabbit tissue to an irritant is the activation of the clasmocytes. The chief source of macrophages in acute inflammation is the hematogenous lymphocytes. (See demonstration no. D35.)

122. The decidual and deciduomal reaction in the pregnant lactating rat. Robert H. Krehbiel, Department of Anatomy, University of Illinois.

Postpartum unilateral pregnancy was interrupted by the concurrent lactation. Instantaneous electrical stimulation of the sterile horn during the normal period of sensitization (4 to 7 days) failed to induce the deciduomal reaction. Prolonged electrical or mechanical (needle stabs) stimulation produced deciduomata without a corresponding decidual reaction in the pregnant horn. Embryonic stimulation induced the decidual reaction after the deciduomal reaction had occurred. In one animal the implantation of an ovum occurred 8 days after the deciduomal response and the pregnancy continued to term. It is concluded that

the sensitization of the endometrium required for the induction of the deciduomal reaction is not the same as that for the decidual reaction and that the formation of these deciduomata is an indication of a specific sensitization and a given stimulus.

57. *Quantitative differences in the human sphincter of Oddi.* Bernard L. Kreilkamp and Edward A. Boyden, Department of Anatomy, University of Minnesota.

Recent cholangiographic studies have revealed the startling fact that one-fifth to one-quarter of the patients so visualized exhibit reflux of contrast media from bile to pancreatic duct. This is never followed by pancreatitis (Leven), but Doubilet has detected pancreatic enzymes in the gall bladder of all such patients and considers this a factor in the etiology of gall bladder disease.

To determine whether there is sufficient muscle around the ampulla of Vater to close the orifice in that 57% of individuals (Baggenstoss) who possess an ampulla, we have extended our maceration studies of the minor duodenal papilla (see current Proceedings of the Physiologists) to the major papilla and have found a well-developed sphincter ampullae in two of twelve specimens. One must conclude, therefore, that in addition to the normally functioning portions of the sphincter of Oddi—the sphincter choledochus, which causes the gall bladder to fill, and the longitudinal fascicles which help erect the papilla—a third portion is sometimes well enough developed to give rise to pathological complications, should it become spastic.

Finally, these maceration preparations have revealed a fourth division of the sphincter of Oddi—namely a group of reinforcing fibers, in the slit-like window of the intestinal muscle layer, that not only reinforce the window at its weakest points but tie the ducts to its margins, thus permitting flexibility while strengthening the choledocho-duodenal junction.

106. *Sexual and asexual nuclear division in a binucleate amoeba.* Benjamin Kropp, Department of Anatomy, Queen's University, Ontario.

In vegetative individuals of *Amoeba diploidea* two nuclei are normally present lying side by side. Each has a prominent karyosome, an outer chromatin-rich region, and a membrane. In the asexual cycle both nuclei elongate, synchronously as a rule. The karyosomes elongate dumb-bell fashion, while the outer chromatin masses tend to accumulate around them. The karyosomes then cross over each other, the outer chromatin meanwhile moving to the poles of the karyosomes. Small granules of karyosome material bud off from the poles but remain attached by filaments to each nucleolus. Continued elongation of the karyosomes results in their eventual separation though they may be held together temporarily by a tenuous filament. The chromatin is concentrated at the poles where it now appears in coarse masses, and shortly thereafter loses its staining qualities. The polar chromatin granules derived from the karyosomes are usually expelled, but are sometimes retained thus giving rise to daughter cells containing four or more nuclei instead of the more usual two. In the sexual cycle two binucleate individuals become enclosed in a common cyst. The two nuclei in each conjugant then fuse by a process resembling synapsis although the chromosomes are not easily identified. After the formation of a single diploid nucleus in each conjugant, the cytoplasm of the two conjugants fuse to form a binucleate individual.

116. *Artificial insemination studies and the functional life of spermatozoa in the female genital tract of the guinea pig.* J. K. Lamar, National Committee Maternal Health, New York, and Carnegie Laboratory of Embryology, Baltimore. (Introduced by C. G. Hartman.)

The primary aim of this study was to determine the length of time spermatozoa can remain in the female genital tract and still be capable of fertilizing ova. It was anticipated from work on persistence of motility of sperms in the female tract that fertilizing capacity would not persist longer than 40 hours. Females were artificially inseminated 2 to 30 hours before heat began (12 to 40 hours before ovulation). As controls, other females were inseminated by the same technics during heat, and still others were allowed single coitus.

The longest time that sperms remained in the female tract before ovulation and still fertilized ova was $22\frac{1}{2}$ hours. This has also been the experience of Young and Soderwall (simultaneous presentation), using different technics of artificial insemination. Fertilizing capacity lasts slightly more than half the time of persistence of motility.

Various methods of artificial insemination were tried, but the one considered best for these studies consisted of injecting into the cervix uteri undiluted sperms from the vas deferens, using a glass speculum and pipette. Sperms in the exudate from the 'plug' obtained by artificial electrical ejaculation of a male guinea pig were also found to be capable of fertilizing when inseminations were made into the cervix.

11. *Control of respiration by the cerebral motor cortex.* Orthello R. Langworthy, Frederick H. Hesser and Rudolf V. Grimmer, Department of Neurology, Johns Hopkins University.

Patients with injury of the efferent fibers from one cerebral cortex show abnormalities in expansion of the chest upon the opposite side. During the period of shock the excursions are decreased. Later the movements on the paralyzed side are influenced by the presence or absence of increased tone. If there is spasticity, the excursions are decreased. In cases where no increase in tone develops, the chest movements are of greater size than on the normal. Attempts to increase the amplitude of respirations voluntarily cause fatigue more easily on the hemiplegic side.

There are rhythmical variations of respiratory excursion associated with bilateral injury of the efferent fibers from the cerebral cortex. These are similar to Cheyne-Stokes respiration, especially in the more marked cases. These patients were not unconscious nor did they have manifest circulatory inadequacy. They showed paresis of the extremities, difficulties in speaking and swallowing, and emotional instability. The administration of oxygen, carbon dioxide, coramine, and aminophyllin tended to decrease the periodicity. This is their effect in other types of Cheyne-Stokes respiration. Hughlings Jackson believed that the respiratory center, released from the control of both cerebral cortices, reacted abnormally to chemical and nervous stimuli.

17. *Proprioceptive impulses and growth of the cerebellum.* O. Larsell, Department of Anatomy, University of Oregon Medical School, Portland.

To study the effect, if any, of proprioceptive stimuli on the growth of the cerebellum, hind limb buds were amputated in tree-frog larvae, opossum pouch-young and rat fetuses. The fibers directly injured do not reach the cerebellum. Presumably the only impulses involved which reach the cerebellum were those relayed through the spinocerebellar tracts, chiefly on one side. Crossed spino-cerebellar fibers, however, present a complicating factor.

The frogs were fixed as near metamorphosis as possible, the rats at term, and the opossums at various stages after operation. The volume of the two sides of the cerebellum was computed from measurements of serial sections. Unoperated controls showed a variation of 1% (rats) and 0.2 to 2.6% (opossums).

Operated frogs, both hind limbs removed, had a cerebellar volume 25 to 29% below that of controls. Opossums (one limb-bud removed) showed a difference of 5.8 to 13.1% between the two sides of the cerebellum. There was considerable difference between sides after amputation of both limb buds, due to variations in the amount of limb bud removed. One cerebellum after this amputation was 41.7% smaller than the control. Rats with one limb bud amputated showed a difference of 10.3 to 24.3%.

69. *A comparative numerical study of the pyramidal tract fibers.* A. M. Lassek, and G. L. Rasmussen, Department of Anatomy, Medical College of the State of South Carolina.

Sections of the medulla just rostral to the motor decussation in mouse, rat, cat, dog and man were stained with protargol and the number of fibers of the pyramidal tract calculated. In our hands, this stain was found to give the best results. The total count obtained for the pyramidal tract of one side for each of the group is as follows: mouse, 32,992; rat, 72,929; cat, 186,047; dog, 285,290; and man, 1,076,431. The square area of the pyramidal tract gives no indication of the size and number of fibers present. Computations on the number of fibers per square millimeter, after individual shrinkage was taken into account, show the mouse to have 480,638, the rat 243,097, the cat 164,142, the dog 81,855, and man 92,235. The fibers of the pyramidal tract of the mouse, therefore, are over five times more numerous for a given square area than in man. On the basis of fiber number per unit area, the pyramidal tract appears to be better developed in the smaller mammals.

95. *The reliability of statistical tests of significant difference between mean body weights of small samples of Wistar male rats from a standardized colony.* F. John Lewis, Department of Anatomy, University of Minnesota. (Introduced by Edith Boyd.)

In experimental studies on rats using small samples of 5 to 10 animals with litter mates or non-litter mates as controls it has become the custom to consider any difference between two means as significant if the probability of its occurrence through chance alone is not more than 5 in 100, i.e., the experimental procedure applied to one sample with the other as control has probably changed

the characteristic being measured. In order to test the reliability of prediction from such statistical procedures applied to rat weights, 232 male rats, which had been used as control animals in various experimental studies, were used. Small samples (five per sample) were drawn from this supply, paired and tested for significance of the difference between means at 3-week intervals over periods ranging from 6 to 54 weeks. Occurrence of these significant differences more often than 5 in 100 would indicate unreliability of the set-up used. None of the differences between the means of samples comparable to the usual litter mate control set-up tested significant. Hence, when differences between experimental and litter mate control animals gives P's of 0.05 or less, it is reasonable to assume that the experimental procedure has caused a significant change in weight.

9. *The Minot pre-somite embryo and the Herzog pre-allantoic embryo as elucidating a still younger stage of human development.* Frederic T. Lewis, Department of Anatomy, Harvard Medical School.

The Minot embryo, with chorionic vesicle (including villi) approximately $9 \times 10 \times 13.5$ mm., has primitive streak, knot, medullary plate and tubular allantois. The volume of its yolk sac is 4.5 times that of its amnion, which has a tent-like peak. The Herzog embryo, with chorionic vesicle (including villi) $1.6 \times 2.4 \times 4.8$ mm., has apparently no trace of allantois. The volume of its yolk-sac is 5.5 times that of its amnion, which has a well-developed amniotic duct. Though this duct, like the blastopore, is 'rudimentary, rare and evanescent' and the amnion has come to arise independently of the trophoblast, we reject Hubrecht's hypothesis of a larval, non-ectodermal envelope, and consider the trophoblast as ectoderm. The Herzog yolk sac has less than one-thirtieth the volume of the Minot yolk sac, yet in proportion to the amniotic cavity it is relatively larger, and leads one to expect a still larger sac in younger stages. It is lined with very thin epithelium except beneath the embryonic shield, where the entoderm thickens to form the protochordal plate of Hubrecht. Between the thin entodermal sac and the chorionic mesoderm is the exocoelom, containing a few cellular shreds and strands embedded in coagulum. Since it is more cellular in the younger embryo, it is reasonable to expect a mesenchymal zone connecting yolk sac and chorion in still earlier stages. (See demonstration no. D36.)

96. *Mechanical features of the articular surfaces of the talus.* J. T. Manter, Department of Anatomy, University of South Dakota Medical School. (Introduced by Wm. H. Waller.)

Measurements of ligament preparations show the line of action of pressure stress at the ankle joint to be coincident with the longitudinal axis of the tibia until the angle of the tibia with the foot is increased by extension to about 105° . Divergence of this line of action from the tibial axis on further extension is dependent on the shape of the posterior part of the trochlea. Cinematic records of walking indicate that such a change in the direction of pressure takes place shortly before the toes leave the ground.

Because of the wedging action of the talus between the malleoli a considerable force is required to establish definitive contact of joint surfaces. An important

buffer mechanism thus comes into action at the moment the heel strikes the ground.

Cinematic records show the occurrence of slight pronation in the subtalar joint soon after the foot is placed on the ground. Since the articular surfaces of the joint form portions of an oblique helicoid, the motion of the talus with respect to the calcaneus in pronation is screw-like, the head of the talus being pressed forward against the navicular bone and turned medially against the cartilage of the plantar calcaneo-navicular ligament. This alteration in the position of the talus favors the support of the talo-navicular joint against the stresses applied when the heel is lifted.

93. Structural changes in the stapes and vestibular window from birth to old age.

John Martin, John E. Karabin and Barry J. Anson, Departments of Anatomy and Surgery, Northwestern University Medical School.

The otic region has been studied both histologically and by wax plate reconstructions in a series of human specimens from the embryo of 7 weeks to the adult of 70 years. The present report is concerned with the form and cartilaginous content of the stapes and fenestral rim in postnatal stages.

The stapes, having attained adult dimensions in midfetal life, exhibits in later stages only the expected degree of individual variation in size. The base of the stapes, once the adult form is attained in late fetal life, remains a bilaminar plate of cartilage and bone, the relative amount of bone increasing as age advances. The rim of hyaline cartilage which lines the vestibular window, relatively thick in the term fetus, undergoes gradual diminution with age; but at no time is the cartilaginous fenestral rim interrupted by osseous invasion. Into this rim the cartilage-covered base of the stapes is fitted, not as would be a plunger in a cylinder, but with the relationship characteristic of a diarthrodial joint. With advancing age the crura become progressively thinner; the head and neck become deeply excavated and, in most specimens, fenestrated. Throughout life the articular surface of the head is covered with cartilage.

10. Effect of extrapyramidal stimulation upon cortically induced movement.

Fred A. Mettler, Harlow Ades, E. A. Culler and Eli Lipman, Department of Anatomy, University of Georgia School of Medicine; Wilmer Ophthalmological Institute, Johns Hopkins Medical School and Department of Psychology, University of Rochester.

Direct stimulation of various portions of the corpus striatum has failed to yield significant results in the past. Nearly all previous experimenters have carried out such stimulation in the absence of motor activity. The present workers undertook to investigate the effect of stimulation of the corpus striatum upon movements already in progress.

Stimulation of various portions of the corpus striatum never gave any results alone but if movement were first induced by stimulation of the motor cortex and then the corpus striatum was excited several new findings were encountered. The basic movement which was studied was phasic forepaw flexion. Stimulation of

the caudate nucleus while a cortically induced movement was in progress resulted in inhibition of the latter beginning with a decrease in amplitude and frequency of the phasic pattern. Inhibition persisted after caudate stimulation had ceased. Stimulation of the putamen gave essentially the same result. Stimulation of the globus pallidus in the course of a cortically induced movement resulted in an holding of the forepaw in a state resembling plastic tonus.

Stimulation of the substantia nigra alone produced an increase in extensor tonus, in conjunction with cortical stimulation a marked tremor resulted. Corpus subthalamicum (alone) stimulation resulted in contraction of the dorsal midline musculature of the opposite side.

101. *A basic dermatoglyphic plan for the primate vola.* Charles Midlo, Department of Anatomy, School of Medicine, Louisiana State University, New Orleans.

Each unmodified primate volar pad is correlated with a primitive dermatoglyphic pattern. There is described a basic plan of pattern arrangement, derived from an analysis of the dermatoglyphics of a large series of primates which included samples of all families and subfamilies (except Indrisinae) of Prosimiae (six genera), Platyrrhinae (nine genera), and Cattarrhinae (fourteen genera, including man).

38. *Homoplastic transplantation of chick metanephric tissue.* John J. Milford, Jr., New York University. (Introduced by C. J. Sandstrom.)

Grafts consisting of one-half metanephroi of 7-day embryos, implanted into the coeloms of 3-day embryos, were removed after 1) 5, 2) 11, and 3) 16 days.

Grafts of series 1), typically recovered from gut mesentery, were ideally vascularized. Generally, the stroma and parenchyma presented a configuration characteristic of equivalently-aged (16-day embryo) kidney, except for an occasional cluster of non-staining, moribund cells of questionable origin. Occasionally nuclei of cells of diverse types and positions displayed amitotic-appearing chromatin. Intense granulopoiesis was frequently observed in loose stroma surrounding vascular spaces.

The grafts of series 2) were incorporated as were those of series 1). In most cases comparison with 18-day embryonic kidneys was favorable. Collecting ducts were frequently occluded with a deep-staining, precipitable material. Granulopoiesis, and the presence and distribution of moribund-cell groups, occurred essentially as in series 1). Invasion by connective tissue or lymphocytes was not evident. A few grafts of this series, although incorporated in the gut mesentery, displayed degenerate tubules and poor vascularity. Granulopoiesis was, however, extensive. Invasive action by lymphocytes and connective tissue was apparent in these instances.

The grafts of series 3), age-equivalents of 2-day chick kidneys, were normal except for occasional moribund-cell groups. Granulopoiesis was intense, and more generally distributed. Collecting ducts contained deep-staining material in their lumina. There was no host reaction. Every evidence suggests that these grafts were functional.

68. *The telencephalic zonal system of the teleost Corydora paliatus.* Ruth N. Miller, Department of Anatomy, Woman's Medical College of Pennsylvania. (Introduced by Hertwig Kuhlenbeck.)

The cell masses of the telencephalon of *Corydora paliatus* show very clearly an arrangement in longitudinal zones which can be recognized and identified as elements of the fundamental longitudinal zonal pattern of the vertebrate telencephalon. These morphological elements have been analyzed and followed with the help of wax plate reconstruction and were found to coincide essentially with the diagram of extremely everted brain pattern previously outlined in *Carassius auratus* and *Cyprinus auratus* by Kuhlenbeck. Apart from the rhinocoele and olfactory bulb the main part of the telencephalon represents a telencephalon medium. The basal zones B2 + 3 and B1 and the pallial zones D1, D2 and D3 were traced. The limiting sulci fd1, fd2, fd3 and fb1 were found developed. The B elements are differentiated into a nucleus basalis pars inferior with medial and lateral subdivisions and a nucleus basalis pars superior with central and dorsolateral subdivisions; the D zones into nucleus epibasalis and nucleus dorsalis, each with central and subependymal portions, and the nucleus lateralis or primordium hippocampi. Of these zones B2 + 3 is the most differentiated. The basal nucleus taeniae and associated pallial structures derived from D3 are well developed. The main fiber connections of these cell masses were studied as far as possible.

33. *Nerve terminals of the human spinal cord.* Jeff Minekler, Department of Anatomy, University of Minnesota. (Introduced by A. T. Rasmussen.)

Block-silver technique, as used by Dr. Barr, shows four main types of end feet (nerve endings) in the human spinal cord: 1) small loops, 0.5 to 1.5 μ (longest dimension), 2) large loops, 1.5 to 4 μ , 3) filamented loops, 1 to 4 μ , and 4) fibrillated bulbs, 1.5 to 5 μ . These may be terminal (boutons terminaux) or pre-terminal (boutons en passant), and may appear regular, thickened, or granular in form. The number per cell is greatest in nucleus dorsalis (Clark's column) and in the ventral gray column. Prolonged formalin fixation diminishes the number of demonstrable endings per cell and increases distortion. The endings can be demonstrated more or less satisfactorily by this technique with formalin fixation as late as 18 hours after death. No end bulbs have been demonstrable by this technique in the newborn thoracic spinal cords so far available, but are evident in small numbers at 10 months. The maximum number per cell is attained by 15 months. Small loops predominate in younger specimens. A relative increase in large loops and fibrillated bulbs occurs with age, but the proportion of filamented loops remains small. Thickened forms appear regularly in small numbers. Certain disease processes produce marked granulation of the end bulbs. Changes in their form in meningitis, poliomyelitis, transverse traumatic myelitis, and in amputation will be indicated.

26. *The effect of hypophyseal implants on the development of the mammary gland.* Warren O. Nelson, Department of Anatomy, Wayne University, College of Medicine.

The original demonstration that crude extracts of anterior pituitary contain some factor or factors which are essential for the development of the mammary

glands in hypophysectomized rats have been extended and a further study has been made of the claim which has been made for the existence of a separate substance involved in the stimulation of gland growth. The production of this substance is said to be increased in animals which have been injected with estrone.

Hypophysectomized female rats and adult male mice were used as test subjects and implants were made of pituitary glands obtained from adult male and female rats which had been injected for varying periods of time with estrone. Material from untreated rats has been implanted in similar test animals and in parallel experiments. The amounts of tissue and the number of implants has varied in individual instances. In some test animals treatment has included injections with estrone during the period of implantations.

Despite the uniformly larger amount of tissue implanted in animals receiving glands from estrone-injected donors there have been no perceptible differences in the degree of mammary stimulation which has been observed at the close of the period of treatment.

58. *A reexamination of the smooth muscle of the ureter.* George H. Paff and Clayton Sharp, Department of Anatomy, Long Island College of Medicine.

Despite the fact that the muscular arrangement within the human ureter was correctly described previous to 1879 by Sappey, present day text books of histology persist in describing it in a manner which is misleading and inaccurate. Mollendorf's reference work in histology is an exception. A reexamination of this subject seemed worthwhile. To that end ureters were dissected microscopically and serial tangential sections were made from the upper to lower end of the ureter proper. It was found that no discrete layers exist. Rather the so-called circular layer represents a felt-work of flat ribbon like bundles which branch and anastomose forming irregular angular spaces between them occupied by connective tissue. From the inner portion of this layer bundles may pass longitudinally to give what is described as the inner longitudinal layer. These bundles may run for some distance linearly but they send broad anastomoses back into the so-called circular layer, and in addition may anastomose with each other. Depending upon the level of the ureter (upper or lower) bundles may pass linearly from the circular layer to form also what in cross-section appears to be an outer longitudinal layer. In the lower part of the ureter many bundles of the circular layer begin to course longitudinally to form a practically complete longitudinal layer.

113. *Sex functions in the senile female guinea pig.* George N. Papanicolaou, Department of Anatomy, Cornell University Medical College, New York City.

Growth of ovarian follicles and oestrous cycles continue up to a very old age in the guinea pig. There is no complete cessation of the sex rhythm, as in human menopause. Instead, there is a gradual retardation in the growth rate of the ovarian follicles, which is reflected in a prolongation of the oestrous cycle intervals with each successive year. A temporary interruption of the cycle (prolonged dioestrus, prooestrus, oestrus, or metoestrus) may appear at any age as a result of some specific cause.

The ovary of the senile animal retains a supply of primary oocytes, though their number is greatly reduced. The follicular activity and the number of growing follicles also show considerable reduction. Follicular atresia, resulting in the formation of atretic corpora lutea, becomes increasingly frequent in the senile animal and tends to replace normal ovulation. The corpora lutea undergo lipid degeneration and then change into amorphous bodies, resembling corpora albicantia. Blood extravasations resulting in the formation of haemorrhagic areas in the ovaries are frequent. Fibrosis and cystic degeneration are the rule.

Fertility decreases rather abruptly and conception becomes very rare after the fifth year. When gestation occurs at such an advanced age it is usually terminated by early resorption or intra-uterine death of the fetuses.

30. *The hypoglossal nerve in human embryos.* A. A. Pearson, Department of Anatomy, Loyola University Medical School, Chicago.

In the early development of the human embryo, the hypoglossal nerve arises from a column of cells in the medulla oblongata. This column is continuous with the anterior gray column of the spinal cord. The hypoglossal cell column in older embryos differentiates into a dorsal and a ventral cell column. The cells of the dorsal column in turn become arranged into a medial and a lateral group. A third group may appear later. The ventral column becomes divided into a dorsal and a ventral group. There is a group of small cells located in close relation to the hypoglossal nucleus.

The hypoglossal nerve was found to have communicating branches which connect it with the first and second cervical nerves, the superior cervical sympathetic ganglion or the sympathetic trunk, the carotid plexus, and the lingual nerve. In a few cases the hypoglossal nerve was observed to send a communicating branch through the root of the tongue to its fellow on the opposite side.

In several embryos, fibers in the descendens hypoglossi turn forward with the hypoglossal nerve. This would indicate that upper cervical spinal nerve fibers course with the hypoglossal nerve. It is quite possible that these fibers may be proprioceptive.

44. *Nerve muscle specificity in regenerated forelimbs of Triturus pyrrhogaster.*

Jean Piatt, Department of Anatomy, University of Vermont. (Introduced by Walter A. Stultz.)

Adult *Triturus pyrrhogaster* were operated on in the following manner. 1) Series A-L, left hand amputated at wrist, 2) series A-R, right hand amputated at wrist and ulnaris nerve divided at origin and completely removed, 3) series B-L, both ulnaris and interosseus nerves divided at level of wrist, 4) series B-R, ulnaris nerve divided at origin and completely removed, interosseus nerve divided at level of wrist. A total of 120 operations were made, thirty per series. The entire flexor musculature of the forearm and hand is supplied almost wholly by the ulnaris and interosseus. The experiments were designed to test the degree of specificity which might exist between a given nerve and its normal muscle field.

All animals were killed at the end of 180 days, a period ample for complete regeneration in all four series. A detailed study was made from serial sections of

all these forelimbs. Generalized summary: a) considering the wide range of normal variation the degree of nerve to muscle specificity is greater than would be expected, b) the general pattern of nerve regeneration is constant and normal, with the exception of the palmar nerve loop, c) specificity in series A-L and A-R is less marked than in series B-L and B-R, d) the specificity manifested by the ulnar nerve for its normal muscle field in series A-L and B-L is the same as in series A-R and B-R where in the latter the distance of regeneration was greatly increased, e) the distribution pattern of a regenerating nerve is not entirely dependent upon the influence of the peripheral stump.

112. Method of recording the electrostatic charge of the living cell. Joseph Pick, Department of Anatomy, New York University College of Medicine. (Introduced by Donal Sheehan.)

The electrostatic charge of the living cell can be tested in two ways: 1) the direct method, using a potentiometer; 2) the indirect methods, based on the conclusion that oppositely charged molecules are attracted, similarly charged ones repulsed. The absorption and storage of the chemical substance can be traced either by means of chemical analysis or by visualization, where dyes have been used. The process of watching the passage of a stain through living human tissue, has been made possible by means of an instrument which combines the principles of the endoscope with those of the microscope.

It has been possible to show that sodium salts and water, whose molecules are positively charged, are attracted by the epithelial cells of the intestinal mucous membrane and subsequently absorbed by the blood capillaries, whereas potassium salts and glucose, whose molecules are negatively charged are absorbed by the lymphatic vessels after passing through the intracellular spaces.

81. Mitotic activity in the pituitary of the white rat following castration. Gerard Roland Pomerat, Biological Laboratories, Harvard University. (Introduced by Alden B. Dawson.)

A series of twenty-five male white rats were castrated at ages varying from 21 to 55 days and killed at intervals of 10 to 33 days following operation. Twenty-one control rats were killed at ages ranging from 28 to 77 days. Eight hours before death and all rats received an intramuscular injection of colchicine in water; the dose being 1 mg. per kilogram of body weight. Frontal sections were cut at 4μ and stained with a modification of Mallory's stain. All the mitoses in ten representative sections from each gland were counted. An average of 345 mitoses were found in the samples from the pituitaries of the castrates, while the normal animals showed 302 each. These include mitotic figures seen in the partes anterior, intermedia, and nervosa. A differential count of the mitoses in the pars anterior showed little difference in the mitotic activity of the acidophiles and chromophobes, but cell division in the basophiles of castrates was nearly three times as frequent as that in the control animals. This would seem to indicate that the significant increase in basophiles observed in the pituitaries of castrated rats could occur in part, at least, from actual division of these cells.

74. *Quantitative studies on the eighth cranial nerve of man.* A. T. Rasmussen, Department of Anatomy, University of Minnesota.

Thirty-eight normal nerves from patients 2 to 60 years old show:

1. Only few unmyelinated fibers and these are largely associated with blood vessels.

2. The number of fibers in the intramedullary portion of the vestibular nerve agrees sufficiently with the number in the large-fibered portion of the extramedullary portion of the eighth nerve that the more uniform and smaller-fibered portion may be regarded as the cochlear nerve, for practical purposes.

3. Nuc. intradicularis nervi vestibularis may contain as many as 5,000 cells and hence is a serious complication in intramedullary enumeration of vestibular fibers.

4. Vestibular fibers vary from 14,000 to 24,000 (average, 18,500).

5. Cochlear fibers vary from 24,000 to 40,000 (average, 31,000), which agrees with the number of spiral ganglion cells (Guild and Hardy). Only 3,000 to 20,000 fibers are reported in the literature.

6. The two components of one nerve tend to depart from the average in the same direction.

7. The right may vary from the left in the same individual by as many as 4,000 vestibular fibers, 5,000 cochlear fibers and 8,500 fibers in entire nerves.

8. In cross section area the vestibular portion is usually the larger. In one-sixth of the cases the cochlear was larger.

9. The funicular pattern varies greatly.

10. Relation of pars intermedia to vestibular fibers may be intimate.

39. *The localization of pigment-producing potency in presomite chick blastoderms.*

Mary E. Rawles and B. H. Willier, Department of Zoology, University of Rochester.

Various regions of blastoderms of head process and definitive primitive streak stages of the Barred Plymouth Rock have been tested for their capacity to produce a barred color pattern in feathers of White Leghorn hosts. The method used was that of grafting small pieces of the blastoderm into the right wing bud of host embryos of 72 hours. It is found for the head-process stage that pieces containing the node, head process or lateral and posterior pieces taken as far away as 0.3 mm. from the primitive pit are capable of producing an area of black nestling down feathers covering all or part of the wing, and often adjacent parts of the breast and back. After hatching these feathers are gradually replaced by juvenile plumage having the barred color pattern of the donor breed. Pieces anterior to the head-process gave negative results. Likewise, pieces containing the node and immediately adjacent parts from blastoderms of the primitive streak stage gave positive results whereas more lateral and posterior pieces (i.e., beyond 0.2 mm. from the pit) gave either negative results or a relatively small area of black feathers.

From these results it is apparent that the blastodermic area capable of producing donor-colored plumage in the host chick coincides with the area known to form nerve tissue in chorio-allantoic grafts. Furthermore, pieces from the center of this area containing the node or head-process (i.e., presumptive floor brain material according to vital staining) produce the greatest effect.

110. *Response of cardiac muscle cells in tissue culture to mechanical and chemical stimuli.* G. S. de Rényi and M. J. Hogue, Department of Anatomy, University of Pennsylvania.

Tissue cultures of chick embryo hearts were used in which the cardiac muscle cells formed a thin transparent plate. With microdissection methods it was found that the large majority of migrating muscle cells do not possess protoplasmic connections with the neighboring cells. The cells are in side to side contact. When one cell was stimulated by mechanical or chemical means contraction occurred in a remote part of the culture, i.e., in the explant. We conclude that protoplasmic connections are not essential in conducting certain types of stimuli. Surface contact between the cells seems to be sufficient to propagate the stimulus from one cell to another for quite a long distance.

117. *Chondriokinesis and spermatid transformation in Orphuella speciosa.* W. R. B. Robertson, Department of Anatomy, State University of Iowa.

Late growth stages of primary spermatocytes show in the cytoplasm, by live-cell methods, four pairs of equal sized, glassy, oblong spheres, two pairs on one side and two pairs on the opposite side of the nucleus. Possibly (?) three divisions have occurred in this material. These bodies tend to attract the mitochondrial threads about them. As the primary cell division approaches the members of each pair of spheroids separate and, at anaphase, there extends between each pair a palisade of mitochondrial threads.

Each secondary spermatocyte receives the ends of four palisades. The head of each halved palisade revolves slightly, tending to wind up its trailer of mitochondria. As the second spermatocyte division comes on there results two (not four) palisades to be cut across.

Each spermatid receives the ends of two palisades, each headed by a 'glassy body.' The double structure revolves slightly on itself to form the nebenkern. As the spermatid transforms, the elongation of the two mitochondrial 'vesicles,' or strands, to form (later) the sheath to the axial filament suggests again an attempt at palisade formation. This phenomenon, together with the double condition and behavior of the centriole and the behavior of other formed elements, nucleus, and parts of the cell as a whole, suggest, possibly, that the transformation of the spermatid may be looked upon as involving an attempt at a third, but abortive, maturation division.

16. *Anatomico-physiological studies on the degeneration of the peripheral nerves.*

William M. Rogers and Horace O. Parraek, Departments of Anatomy and of Physiology, College of Physicians and Surgeons, Columbia University.

The sciatic nerve in rats and cats was cut at the level of the neck of the femur. Tests were made with induction shocks to determine the length of time during which function persisted. Action potentials of the severed and control nerves were compared. No action potential was obtained after stimuli applied to the peripheral end failed to produce a contraction of the muscles which it supplied. The nerve fiber and its motor end organ ceased to function simultaneously, therefore muscular contraction was used as an index for nerve function.

The extent of contraction obtained by maximal stimuli decreased during the latter half of the survival period, indicating progressive physiological failure of individual nerve fibers.

Twelve sciatic nerves from cats and twenty-eight from rats were studied. A variety of methods were utilized to afford a more accurate comparison of degenerating and normal nerve. The following proved helpful:

1. Frozen sections examined under the polarizing microscope.
2. Frozen sections stained with Sudan III, Scharlach R and osmic acid for changes in the myelin.
3. Pieces of nerve and muscle stained with gold chloride for axones and endings.
4. Frozen and paraffin sections stained with reduced silver nitrate to illustrate axonal structure.

The progressive loss of function in the severed nerve corresponds to successive degeneration of individual fibers.

105. Effect on the embryo of x-radiation of frog sperm. Roberts Rugh, Department of Zoology, Columbia University.

Using 200 K.V. x-ray source, frog spermatozoa were exposed to varying doses from 15 r to 65,000 r before they were used to inseminate normal eggs. Some abnormal embryos were produced when the spermatozoa were exposed to as little as 15 r, much less than the average clinical dose for carcinogenous tissue. At 500 r about 50% were abnormal; at 1000 r few hatched and at 10,000 r all died before or during neurulation and there was no hatching. When the dose was extended beyond this to 65,000 r all hatched. The tadpoles showed slight dorso-ventral widening of the tail; telescoping and flexing of the body; and slight reduction in size. Otherwise these tadpoles appeared normal. They may be haploids.

The abnormalities that appear are exogastrulation, spina bifida, and oedema, indicating a disruption of the embryo at or near the period of gastrulation. At no radiation level was the rate or pattern of early cleavage altered as compared with the controls, indicating a passive relation of the sperm nucleus to this process. With increasing doses the probability of a direct hit (or hits) involving points, sections, or whole chromosomes, is increased and at 10,000 r the sperm nucleus is apparently able to take just enough part in embryonic development to act as a contributing lethal, while above 30,000 r it can function only as a parthenogenetic agent but with 100% effectiveness. (See demonstration no. D42.)

35. Embolic lesions in the opossum brain. Ernst Scharrer, The Rockefeller Institute for Medical Research, New York. (Introduced by C. V. Morrill.)

The blood vessels of the opossum brain are not only anatomically, but functionally true end arteries. Embolic lesions resulting from injections of lycopodium spores into the carotid artery, indicate that there are no areas in the brain nourished to such a degree by overlapping capillary loops as to prevent complete ischemia. The ischemic territories correspond in shape and extent to the area of supply of an occluded vessel. There are no more capillaries than

necessary to insure the supply of the nervous tissue. The diffusion sphere of each capillary seems to overlap little or not at all the sphere of any neighboring capillary. The fairly equidistant distribution of the capillaries in the opossum brain is to be interpreted as the anatomical expression of the vascular economy. It is very improbable that in the opossum brain capillaries are closed and opened in response to the changing requirements of activity and rest, as occurs in muscle. The prompt ischemic reaction of the nervous tissue in cases of occlusion of blood vessels by emboli makes it appear unlikely that under normal conditions any capillary in the opossum brain is ever closed for any length of time.

59. The spatial distribution of calcium and magnesium in contracted skeletal muscle. Gordon H. Scott and Donald M. Packer, Department of Anatomy, Washington University School of Medicine, Saint Louis.

Sections of frozen and dehydrated muscle prepared by a modification of the Altmann-Gersh method were vacuum-distilled in an electron microscope and heated to the emission point of calcium and magnesium. If the sections were placed on a barium and strontium coated cathode, magnesium and calcium were activated selectively to strong emission. Sodium and potassium are not revealed by this modification of the electron microscope method. Photographs will be presented which show magnesium and calcium in the contraction bands of the fiber and not in other parts of the muscle cell. There is little or none of these elements detectable in the connective tissue of muscle.

23. Growth and development of white rats under treatment with thymus extract (Hanson) for four successive generations. Albert Segaloff and Warren O. Nelson, Department of Anatomy, Wayne University, College of Medicine.

Rowntree and his co-workers have reported that treatment of successive generations of white rats with thymus extract (Hanson) led to a remarkable increase in the rate of growth and differentiation which became more marked with each succeeding generation. This study was undertaken in an attempt to reproduce this phenomenon.

Thymus extract (Hanson) was very generously supplied fresh every other week by Doctor Hanson. The extracts used showed at all times appreciable quantities of iodine reducing substances which are said to be associated with the titer of activity.

All the animals were kept under the same conditions and received our normal colony diet of Bal-O-Ration and water ad libitum plus greens in the form of lettuce daily.

No significant differences were noted in the rate of growth, age of opening of the ears or eyes, eruption of the teeth, descent of the testes or canalization of the vagina. Nor were any significant differences in organ weights noted at 70 days. These observations were uniform for the control and experimental groups in each of the four generations. Each of these groups beyond the first generation contained at least fifty animals.

This study is being continued through further generations.

94. *The vascular pattern of the mouse thymus.* Christianna Smith, Betsy D. Conant, Eleanor G. Sayer, Department of Zoology, Mount Holyoke College.

The vascular pattern of the mouse thymus differs from that usually given for mammals and diagrammed by Watney in 1882. In the mouse one commonly finds that the main artery enters and the vein leaves in the region of a hilus on the median surface. The artery and vein run together in the medulla with many branches but the larger medullary veins seem more prominent. At the region of the junction of the cortex and medulla arterial capillaries run vertically to the surface, continue as capillaries underneath the capsule for a short distance and then return as parallel venous capillaries to the veins of the medulla. The length of these cortical capillaries shortens with the decrease in width of the cortex in involution. In markedly diminished thymuses which are very thin and leaf-like, the regularly arranged capillaries of the cortex are no longer apparent and the arteries are tortuous.

108. *The regeneration of sensory olfactory epithelium in the frog.* C. G. Smith, Department of Anatomy, University of Toronto.

Introduction of a solution of 1% zinc sulphate into the nasal cavity of frogs was shown to cause complete degeneration of the olfactory epithelium. To study the process of regeneration, one side of the nose was treated in a series of animals and specimens were examined at suitable intervals thereafter up to 3 months. The results are as follows. At the end of 3 days a single layer of flattened cells covers the denuded surface. During the succeeding 4 weeks the epithelium increases in thickness until it is six to eight cells deep. Differentiation then begins so that by the end of 3 months the epithelium has completely regenerated and differs in no way from that of the untreated side. Both the primary olfactory sensory neurons and the supporting elements regenerate.

115. *Spermatozoa viability in the genital tract of the guinea pig.* Arnold L. Soderwall, William C. Young and Richard J. Blandau, Department of Biology, Brown University.

A recent investigation by Blandau and Young on the effect of ovum age on development in the guinea pig suggested a study on the effect of delayed fertilization on the fertilizing capacity of spermatozoa and on the development of the embryo.

Animals with known reproductive records were artificially inseminated shortly before an expected heat period and observed continuously until heat occurred. In this way the interval between the time of insemination and the beginning of heat was determined. Since ovulation usually occurs about 10 hours after the beginning of heat, the interval between the time of insemination and fertilization for each animal was considered as being 10 hours longer.

One hundred and fifty-two females were inseminated 1 to 122 hours before heat. Seventy-four control animals were inseminated during heat. In no instance was the fertilizing capacity retained longer than $22\frac{1}{2}$ hours. The frequency of fertilization showed a marked decrease only after 18 hours. Since the percentage of fertile inseminations then decreased rapidly and the frequency of

abnormal pregnancies was not significantly greater in the experimental than the control group, an all-or-none capacity of spermatozoa to effect fertilization under these conditions is indicated.

Thirty-three animals failed to come into heat following insemination. Nevertheless, pregnancy occurred in twelve.

34. The lenticulostriate and lenticulo-optic arteries. Othmar Solnitzky, Georgetown University Brain Research Institute, Georgetown University School of Medicine.

In 100 human brains the middle cerebral artery was injected with India ink and its basal branches followed in their intracerebral course by actual dissection.

The basal branches of the middle cerebral artery were given off while the artery crossed the anterior perforated space. They entered the brain substance just in front of the attachment of the temporal lobe.

The branches vary in number from six to eighteen. They vary in diameter from 0.5 to 1 mm. The smaller branches leave the artery at right angles, while the larger branches come off at acute angles. All the basal branches converge from their origin upon the point of entrance where they are concentrated in a small area at the base of the external capsule, between the claustrum and the putamen. From this point they pass laterocaudally to supply the greatest part of the putamen and the caudate nucleus, the lateral half of the globus pallidus, and the whole of the internal capsule above the level of the globus pallidus.

In none of the brains was any basal branch found that could be singled out as the largest. In no case was the most lateral of the basal branches the largest. In no case was any evidence found that the thalamus is supplied by basal branches of the middle cerebral artery.

Since there is no evidence whatever of the existence of a lenticulo-striate artery or artery of cerebral hemorrhage and of a lenticulo-optic artery, these term should be eliminated from anatomical nomenclature.

86. Histological reactions of the head furnishings of the White Leghorn capon to urinary androgens applied to the surface of the comb. David Soloway, Department of Anatomy, Laboratory of Histology, Temple University School of Medicine. (Introduced by John Franklin Huber.)

The combs of twelve capons were anointed with urinary androgens and the combs, wattles and earlobes removed for microscopic study at intervals from 3 to 188 hours. The number and distribution of blood vessels in the head furnishings of other capons and cocks were studied with injection masses.

A surprisingly large network of dermal capillaries persists in the atrophic capon comb and wattles and these vessels dilate within the first few hours following the anointment. There follows a proliferation of new capillaries, more pronounced toward the attached portion of the comb and wattles and particularly in the dermis surrounding the bases of the surface papillae. About 15 hours after the first application, the ends of the large bundles of collagenous fibers, in the region where the dermis and central core are in apposition, begin to fray and split into smaller bundles and constituent fibrillae, the latter showing an

affinity for mucin stains for the first time. Finally, there is a rapid deposition of intercellular and interfibrillar mucin, establishing the typical subcutaneous layer.

The earlobes respond in a totally different manner, their growth being caused by a proliferation of large intertwining bundles of collagenous fibers with no increase in the number of capillaries and no mucin demonstrated at any time in the earlobes of either the cock or the capon.

15. *The adjustments of cutaneous nerve endings during growth.* Carl Caskey Speidel, University of Virginia.

The cutaneous nerve endings of myelinated fibers undergo marked changes during growth. These changes have been directly watched in living frog tadpoles for prolonged periods. A complete history of the same cluster of endings emerging from a myelinated fiber at a node of Ranvier has been recorded in each of two tadpoles from shortly after the animals hatched until they attained full size.

Among the features observed were multiplication of endings by branching, loss of some endings by retraction or by degeneration, growth in length of some new endings of as much as 200μ , variations from day to day in exact position of the terminal buds at the basement membrane of the epidermis, variations in the shape of the terminal swelling from olive or sphere (resting type) to growth cone (advancing type), or to retraction club (retreating type), or to swollen watery sphere (irritated or degenerating type), and elimination of aberrant deep non-cutaneous endings which originated during the early haphazard growth of branches in all directions.

The cluster of endings in each case belonged to a myelinated fiber along which several additional myelin segments developed terminally during the period of observation. One case also exhibited wallerian degeneration ultimately. A neurilemma cell which had started to transfer to one of the endings under observation retreated as degeneration set in.

29. *Action of esterone on the sex organs of immature male cats.* William F. Starkey, Department of Biology, University of Pittsburgh. (Introduced by John C. Donaldson.)

Esterone was injected intramuscularly over varying periods into ten immature male cats. Seven males, six of which were litter mates of the experimental animals, served as controls. All experimental animals showed hypertrophy of the glandular epithelium of the prostate. In addition, there was metaplasia of the epithelium of the urethra without leucocytic infiltration. (See demonstration no. D47.)

60. *Growth and differentiation of connective tissue as observed microscopically in the living rabbit.* Mary L. Stearns, Department of Anatomy, University of Pennsylvania. (Introduced by E. R. Clark.)

The development of connective tissue in the living animal was studied by daily microscopic observations of its ingrowth in a series of transparent chambers inserted in rabbits' ears.

The new growth began within a week after operation, when fibroblasts invaded the table migrating inward at an average rate of 0.19 mm. a day. Fibers appeared 4 or 5 days later. They were laid down at approximately 0.27 mm. a day, and covered the table, 6.0 mm. in diameter, 3 weeks after operation.

There was a characteristic tempo and pattern of fiber formation. However, both varied slightly in different chambers and in different regions of the same chamber. Both were modified by the presence of epidermis. The rates of fibrous and vascular development appeared correlated, although actual vascularization was not essential to fiber formation.

These observations indicated that the fibroblast is essential to fibrous development, and that its activity is intimately associated with the actual formation and orientation of the fibers.

No evidence was found to indicate that fibers formed as continuations of preexisting fibers, by a direct transformation of fibrin, or through the activity of any cell but the fibroblast.

Tension influenced the rate, amount, and direction of fiber formation.

109. Changes in the structure of skeletal muscle at the time of its first visible contraction in living rat embryos. William L. Straus, Jr., Department of Anatomy, The Johns Hopkins University.

As one phase of an investigation of the anatomical and physiological changes that occur in the development of fetal skeletal muscle, living rat embryos were stimulated. Visible contractions of the arm musculature followed electrical stimulation through the skin of the embryo during the sixteenth day of gestation. Embryos of the fifteenth day gave no visible response to the same procedure.

Comparing the muscles of these two stages: The fibers of the 16-day embryos in general are larger and contain more myofibrils, especially those of advanced morphology. Presumably important extramuscular differences relate to the more advanced organization of the connective tissue and to the more conspicuous tendency toward articular cavitation in the 16-day embryos.

Muscular contraction can be produced by stimulation of nerves in the sixteenth day. The nerve endings at this time are epilemmal and of relatively immature form.

It is likely that the differences in structure of the muscle fiber between 15-day and 16-day embryos are insufficient to account entirely for the differences in visible physiological response to stimulation. Explanation therefore seems partly to reside in extramuscular factors.

85. Effects of ovarian ear grafts in castrate male mice compared with effects of combined estrogen-androgen injections. M. T. Strong and R. T. Hill, Department of Anatomy, Indiana University School of Medicine.

Ovaries grafted into the ears of castrate male mice kept at low temperatures secrete sufficient androgen to restore the castrate type of seminal vesical and prostate (combined weight 16 mg.) to the normal weight of approximately 150 mg. This weight is maintained indefinitely without further increase.

Assays of several artificial androgens have been made in an attempt to simulate the effects of the ovarian androgen. In doses given, testosterone, testosterone propionate, and androstenedione produce steady increases in accessory weight without the early plateau produced by the ovarian androgen.

Testosterone propionate (Oreton), injected subcutaneously on alternate days in doses of 0.125 mg. in 0.05 cc. of oil, gives the following accessory weights, 5 days, 78 mg.; 10 days, 115 mg.; 15 days, 175 mg.; 20 days, 251 mg.

If, however, castrate males are given the same doses of testosterone propionate to which has been added 1250 i.u. of Progynon B, the accessory weights are, 5 days, 62 mg.; 10 days, 116 mg.; 15 days, 131 mg.; 20 days, 120 mg.

The effect of testosterone propionate was inhibited by the addition of Progynon B producing a plateau comparable with that resulting from ovarian ear grafts.

These data suggest that the early plateau in the ovarian ear graft curve may indicate that the androgens are held in check by the estrogenic activity of the grafted ovary.

25. Hypertrophy of the dog suprarenal gland following unilateral suprarenal-ectomy. C. A. Swinyard and H. D. Bruner, Departments of Anatomy and Physiology, Medical College of the State of South Carolina. (Introduced by A. M. Lassek.)

Unilateral suprarenalectomy was performed on seven male and seven female dogs. In three of the male dogs and four of the females the right gland was surgically excised while in the remaining animals the left gland was removed. The dogs were sacrificed at periodic intervals during the postoperative period (7 to 290 days) and the second suprarenal extirpated.

On the basis of Baker's ('37) conclusion that there is no significant difference in the size of the normal right and left suprarenal gland of the dog, hypertrophy was determined by comparing the surgically excised gland with the one removed at autopsy. In thirteen of the fourteen animals operated upon, the gland extirpated at autopsy was larger than the one taken out surgically. The hypertrophy varied from 8.4% to 22.8% and averaged 14.6%. In all of the glands removed at autopsy the cortical portion of the gland was larger than the cortex of the normal gland of the opposite side. The cortical hypertrophy varied from 3.2% to 29.9% and averaged 15.6%. The volume of the medulla varied widely and apparently did not participate in the gland enlargement.

2. Anomalous epithelial structures in the myocardium of pig embryos. George F. Sykes, Department of Anatomy, Tufts College Medical School.

In sixty of 300 pig embryos examined histologically, both vesicular and solid epithelial growths have been observed in striking relation with the myocardium. The range of these bodies extends from the endocardial cushion, through the morphological ventral wall of the ventricle, into the sinoatrial region. The cystic bodies are composed of simple columnar epithelium. Of the solid bodies, some clearly exhibit their epithelial character, while others present a hyaline appearance. The question of histogenesis is considered together with possibilities of interference with normal development of the cardiac valves and the atrio-ventricular bundle. (See demonstration no. D49.)

66. *A method for studying the mammalian pulmonary alveolus during respiration.*

Robert J. Terry, Department of Anatomy, Washington University School of Medicine.

A window has been constructed which fitted into an intercostal space in an anaesthetized cat permits observation of the superficial alveoli. The problems relative to the selection of the site, maintenance of an air free pleural cavity and illumination will be discussed, and a report of observations will be made on the changes of form and volume of the alveolus and the behavior of the alveolar aperture.

51. *Qualitative differences in the reaction of the large phagocytic mononuclear cells to subcutaneous injections of the phosphatides and cerebroside of beef brains.* Edna H. Tompkins, Department of Anatomy, Vanderbilt University School of Medicine.

The acetone insoluble lipoids of beef brains were fractioned by differential solubilities. The purified products were injected subcutaneously in guinea pigs and rabbits. Material obtained from the site of injection 3 to 5 days later was stained supravitaly with neutral red.

The chemical fractions were found to be biologically divisible into three groups, based on the specificity of the reactions of the large phagocytic mononuclear cells. All of the phosphatid fractions gave rise to infiltrations composed almost entirely of markedly phagocytic mononuclears which showed no evidences of toxic injury. The character of the phagocytic mononuclears, however, in the areas injected with the ether soluble phosphatides, i.e., the lecithins and cephalins, differed characteristically from that of the phagocytic cells in the areas injected with the ether insoluble phosphatides, i.e., the sphingomyelins. While the cells in the areas injected with either of the ether soluble phosphatides were large and actively phagocytic and morphologically alike, those in the areas injected with the ether insoluble phosphatides were still larger, and contained characteristic vacuoles of greater size which exhibited a marked uniformity of both size and color. The reaction of the large mononuclear phagocytes of the connective tissues to the cerebroside, on the other hand, was secondary to extensive infiltrations with neutrophilic polymorphonuclears, and exhibited the varied and often degenerative characteristics of the large mononuclear cells in infectious areas.

13. *Neuron transformation from the bipolar to the unipolar phase, as observed in the chicken gasserian ganglion.* R. C. Truex, Department of Anatomy, Columbia University. (Introduced by S. R. Detwiler.)

The dominant cell types in this sensory ganglion are the unipolar neuron, although bipolar and transitional stages are found to form 1 to 2% of the cells. Studies of such neurons in intermediate stages between the bipolar and unipolar phase, cast a new and interesting light upon the method of transformation.

The generally accepted belief that the unbranched stem, or axon of a unipolar neuron is the equivalent of the two processes of a bipolar neuron may be questioned. It does not follow from this investigation that an actual fusion

of the two approximated processes occurs. Instead of such fusion, there appears to be a gradual protrusion and elongation of the cytoplasm in the region between and at the site of attachment of the two approximating processes.

The involved cytoplasm marks the region of the future axon hillock, and is itself converted into the undivided portion of the unipolar process, or stem. At the apex of this stem (transformed cytoplasm) one observes the two original opposi-polar processes of the bipolar phase.

123. *The origin of the vaginal blood in the pregnant albino rat. The 'placental sign.'* John H. Venable, Department of Anatomy, Harvard Medical School. (Introduced by George B. Wislocki.)

Blood appears in the vagina of the albino rat as early as the eleventh day of pregnancy and is added to each day through the sixteenth. Intra-uterine extravasation from decidual vessels in relation with the ectoplacental cone at the lowest implantation site of each horn is the source of this blood. Similar extravasations occur at each implantation site, but do not contribute to the vaginal contents because of occlusion of the lumen. Because of antimesometrial implantation the lumen is patent mesometrially between the sixth and eleventh days. On the eleventh day the ectoplacental cone makes contact with the mesometrial uterine wall and thus obliterates the lumen. This bipolar attachment and occlusion exists from the eleventh to fifteenth days. On approximately the fifteenth day the antimesometrial attachment is interrupted and the embryo remains until term attached only mesometrially by the definitive placenta, and the lumen is again patent but only around the antimesometrial pole. Intrauterine extravasation begins on the eighth day, but does not become extensive until the tenth. The mesometrial lumen is then narrow and contains a mass of blood near the cone, factors which make it unlikely that blood from upper sites can descend into the vagina. The blood trapped above the lowest site is reabsorbed before the lumen reopens mesometrially, there is no increment in vaginal blood at that time.

50. *Effect of hypophysectomy on the blood picture of the rat.* Erwin P. Vollmer, Albert S. Gordon, Irving Levenstein and Harry A. Charipper, Department of Biology, Washington Square College, New York University.

Following complete hypophysectomy in 150 to 230 gm. rats the red cell count drops steadily from a normal value of 8.0 to 8.6 million per cubic millimeter to 4.5 to 5.2 million per cubic millimeter. The latter count is attained approximately 2 months subsequent to the operation and is maintained at this level for at least another month. Correspondingly, the hemoglobin drops to a value 30 to 40% below normal. Young circulating red cells (reticulocytes) are found in fewer numbers in such animals than in the untreated or operated controls. The bone marrows of rats hypophysectomized for 3 to 4 months exhibit considerable hypoplasia. In control starved rats showing weight losses comparable to that occurring after hypophysectomy the red cell count and hemoglobin do not decrease but actually increase above normal values. Partially hypophysectomized animals and control animals subjected to sham operations without the removal of the hypophysis show a temporary anemia after which the normal red cell count is soon attained.

Although an increase in the total white cell count and a relative lymphocytosis occurred soon after hypophysectomy, similar results were obtained in the control operated animals.

It may therefore be concluded that hypophysectomy in the rat results in an anemia which is not due to low intake of food or to trauma incident to operation. The erythropoietic system is depressed. The nature of this depression is yet to be ascertained.

27. The effect of synthetic androgens on the mammary gland of the monkey.

G. van Wagenen and S. J. Folley, Department of Obstetrics and Gynecology, Yale University School of Medicine.

Pre-adolescent female monkeys were chosen at a time when the mammary gland was beginning its peripheral extension and elaboration (1800 to 2500 gm.). A control mammary gland and both ovaries were removed. The control glands made it possible to arrange the animals in a series so that the effect of testosterone propionate was investigated not only on glands consisting of a few unbranched ducts, but also on glands with alveoli and beginning lobule formation and several stages in between. Injections of 10 to 200 mg. per week were continued for 10 to 70 days. A definite secretory response was obtained from every type of gland receiving testosterone propionate. Colorless serous fluid could be expressed from the nipples and the entire duct systems of all glands were greatly distended. The absence of connective tissue proliferation and the fact that the terminal buds were thin walled and ballooned appeared to rule out growth of the gland by extension. On the other hand, pre-existing lobular tissue was increased in amount and histological examination showed the alveolar elements to have proliferated and partially organized.

Of three other androgens given at high dose levels dehydroandrosterone and Δ 5-transandrostendiol caused very little secretion when given in the absence of lobular tissue and androsterone gave only a slight reaction. Apparently, by varying the androgen administered it is possible either to elicit or avoid a pronounced secretory response within the mammary gland.

73. The origin, course and terminations of the secondary pathways of the trigeminal nerve in primates. A. Earl Walker, Department of Neurology and Neurosurgery, The University of Chicago. (Introduced by S. Polyak.)

In a series of six monkeys (*Macacus mulatta*) lesions were made in the spinal and main sensory nuclei of the trigeminal nerve. The brains were treated according to the Marchi technique and sectioned serially.

Degenerated fibers from the spinal nucleus of the trigeminal nerve cross the midline in the lower part of the raphe to reach the most inferior and lateral part of the medial lemniscus. Some of the degenerated fibers follow the course of the spinothalamic tract through the brain stem and terminate in the medial portion of the ventral posterior nucleus of the thalamus (nuclei ventralis posteromedialis and ventralis posterolateralis). The degeneration does not reach the medial part of the arcuate nucleus.

From the main sensory nucleus of the trigeminal nerve degenerated fibers pass across the midline and turn rostrally just dorsal to the medial lemniscus. These fibers follow the course of the medial lemniscus through the brain stem to the thalamus where they end in the lateral part of the nucleus ventralis posteromedialis. A second and smaller group of fibers, some crossed but the majority uncrossed, pass cephalad through the dorsolateral part of the reticular substance. In the mesencephalon they lie just lateral to the third nerve nuclei and terminate in the medial part of the arcuate nucleus of the thalamus (nucleus ventralis posteromedialis).

14. *The production of artificial 'tumors' in the brains of cats.* James W. Ward and Sam L. Clark, Department of Anatomy, Vanderbilt University School of Medicine.

Under aseptic conditions small grease-cup-like plugs were screwed into trephined holes in the skulls of a series of cats. The dura under the holes was removed. The cups extended through a hole in the skin and were filled with a mixture of beeswax and lipiodol. The top of the cup was screwed down daily, forcing wax in small amounts into the cranial cavity. The wax was of such consistency as to form a ball in the brain substance. Plugs were placed on most of the accessible points on the cerebrum. Observations were made daily on the placing reactions and the general health. The optic discs were examined at intervals. At death the animals were injected with 10% formalin and the brain preserved for histologic study. 'Tumors' on the anterior half the cerebrum have caused a selective loss of placing reactions in one or both opposite extremities. Remission of these symptoms have occurred. Occasionally mild motor seizures have followed the injection of small amounts of wax, but true Jacksonian convulsions have not appeared. Cerebrospinal fluid block with development of changes in optic discs, lethargy, and death has been produced. X-ray pictures of the head were taken of the animals upon the appearance of symptoms. Without infection the 'tumors' tend to become encased with a connective tissue capsule continuous with the dura.

41. *The influence of normal and induced metamorphosis on hind limb regeneration in Rana sylvatica.* A. Emerson Warren and Campbell M. Bower, McMaster University, Hamilton, Ontario.

The significance of Speidel's experiments to theories explaining the loss of regenerative power in anurans has prompted the extension of similar investigations covering a greater range of developmental stages. Linear growth of normal and regenerating limbs has been studied under conditions of normal and induced metamorphosis at eight stages in limb development. Several hundred larvae were measured at 3-day periods following the amputation of parts of the limb. Metamorphosis was hastened at successive intervals by treatment with a 0.005% diiodotyrosin solution.

The results of our experiments show that no regeneration occurs if di-iodotyrosin is administered before, during, or immediately after the amputation. If it is administered after the proliferative phase is established, however, a marked increase in regenerative rate occurs. These observations concur with those of Speidel. Thus metamorphosis is not in itself an inhibiting factor of constant value. Rather, its effect on regeneration varies from complete inhibition to rapid acceleration, depending upon the extent of regeneration which has preceded the metamorphic climax. In all developmental stages the change in the effect occurs abruptly, the critical point being approximately 5 days after the amputation. This appears to coincide with establishment of the proliferative phase of regeneration.

These results indicate that the regenerative capacity is influenced to a much greater extent by the amount of thyroid secretion normally present, than by the degree of limb differentiation.

84. Sperm formation induced by androgens following anterior pituitary removal.

L. J. Wells and M. D. Overholser, Department of Anatomy, University of Missouri.

We have killed 108 hypophysectomized ground squirrels (forty-two immature, sixty-six adult) and studied reproductive organs. Eighty-five lacked spermatozoa at hypophysectomy. One hundred survived for 30 to 184 days.

Sixty-eight of seventy-five injected animals received testosterone or testosterone propionate daily for 25 to 32 days preceding autopsy (seven for 12 to 24 days), daily dose being 0.5 or 1.0 mg. At first injection, seventy-one treated males lacked sperm (determined by unilateral orcheectomy in thirty-six adults, and testis palpation). Thirty-three were hypophysectomized controls.

Hypophysectomy success in each was determined from serial sections of sella turcica. Fragments of nervosa, intermedia and tuberalis were usually present. Volume of anterior pituitary fragments (when present) was determined by Edinger's projection apparatus and planimeter.

Thirty of thirty-three controls were killed during months of sperm expectancy. Twenty-one of these exhibited no anterior pituitary fragment; nineteen showed no sperm in fresh smears and stained sections of testis, one showed few and another exhibited many.

Fifty-one animals received testosterone. Thirty showed no anterior lobe fragments; twenty-one of these had spermatozoa.

Twenty-four received testosterone propionate. Fourteen showed no anterior pituitary fragment, and each had spermatozoa.

Seven of twenty-six injected animals with pars anterior fragments failed to show sperm.

We conclude that testosterone and testosterone propionate can induce sperm formation in immature and adult ground squirrels in the absence of the anterior pituitary.

75. *The VIIIth nerve and its associated ganglion cells and inner ear sensory areas in Sphenodon.* Jean K. Weston, Department of Anatomy, Temple University School of Medicine.

The material comprised two series of *Sphenodon* stained with iron hematoxylin, one with the central nervous system in situ.

Measurements carried out on the latter showed that the total inner ear sensory area $2.98 \pm \text{sq.mm.}$) was approximately one-fourth that of man; 60% being pars superior (60% macular type—40% canal type) and 40% pars inferior (97% saccular type—3% cochlear type) in position.

Projecting each inner ear sensory area onto its associated ganglion cells, the crista anterior occupied the most rostradorsally lying ganglion cells of the VIIIth nerve, followed in order, passing caudoventrally, by the crista externa, macula utriculi, macula sacculi, crista posterior (and papilla neglecta), macula lagenae, and papilla basilaris cochleae. Those ganglion cells related to the ramus anterior of the VIIIth nerve tended to be intracranial in position as contrasted with the intraotic position of the ramus posterior ganglion cells.

The localization of the sensory areas within the VIIIth nerve nuclei of the medulla oblongata appeared to be, from dorsal to ventral, papilla basilaris cochleae, macula lagenae, macula sacculi, crista posterior (and papilla neglecta), crista anterior, crista externa, and macula utriculi. The material did not permit precise rostrocaudal localization of these sensory areas within the medulla oblongata, although earlier comparative reptilian studies of this region (Weston, '36) noted but slight relative dorsoventral shifting of the incoming VIIIth nerve fibers after their entrance.

45. *A radiological study of visceral and respiratory activities of the fetus in amnio.*

W. F. Windle, R. F. Becker, E. E. Barth and M. D. Schulz, Departments of Anatomy and Radiology, Northwestern University Medical School.

Avoiding anesthetics and surgical procedures, we pierced the abdominal and uterine walls of thirteen guinea pigs between mid-pregnancy and term (9 weeks), withdrew 0.4 to 1.0 cc. of amniotic fluid and replaced it with colloidal thorium dioxide or hydroxide. Radiograms were obtained within a few minutes and at various intervals until birth.

During the last 2 weeks of gestation thorium appeared in the stomach in 1 to 4 hours. By 24 hours much fluid had been drunk. By 40 hours, thorium had become concentrated in the small and large intestines where segmentations were clearly defined. Near term passage through the entire gastrointestinal tract, defecation and meconioophagy in amnio were demonstrated. In fetuses of the sixth and seventh weeks, passage of thorium was slower. It appeared in the stomach at 24 hours, small intestine at 40 hours, large intestine at 60 hours, but even after 14 days meconium had not passed. We conclude that amniotic fluid is swallowed as early as the fortieth day and soon thereafter material traverses the intestines by peristalsis, concentrating in the large intestine; meconium passes a few days before birth, mixes with the amniotic fluid swallowed.

In no instances, did thorium appear in the fetal lungs under normal conditions. But when asphyxia prevailed, e.g., mother breathing nitrogen, the fetuses 'breathed' fluid and lung shadows were seen in the radiograms. (See demonstration no. D54.)

71. *The fiber connections of the accessory olfactory bulb of the rabbit (Lepus cuniculus)*. M. Wharton Young, Department of Anatomy, Howard Medical School.

The afferent connections of the accessory olfactory bulb by way of the vomeronasal nerve are well known but less is known of its efferent fibers. A study of the fiber connections has been attempted with Weil, silver and Marchi stains. The Marchi series were prepared following partial and complete ablations of the accessory bulb. The animals showed no abnormalities subsequent to operation except frequent and repeated sniffing. The majority of the fibers from the accessory olfactory bulb emerge in a dorsal and ventral pedicle at the lateral aspect of this bulb. These emerging fibers then assume a superficial position in the lateral olfactory tract. These fibers are smaller in caliber and have thinner myelin sheaths than the fibers from the olfactory bulb. Caudal to the plane of the accessory bulb the fibers from the same tend to aggregate in the dorsal and ventral part of the lateral olfactory tract and this arrangement has been correlated with the splitting of the pars externa of the nucleus olfactorius anterior, pars lateralis.

DEMONSTRATIONS

Titles with designation "Dm-" are motion picture demonstrations; in the program of the meeting they follow the general demonstrations.

D1. The vascular structure of the aortic and carotid bodies in the adult dog.

William H. F. Addison and J. H. Comroe, Jr., University of Pennsylvania.

Photomicrographs and sections stained by different connective tissue techniques.

D2. Neuronophagia in the cerebral cortex in senility in man and the mouse.

Warren Andrew, Department of Anatomy, University of Georgia School of Medicine.

Slides and photomicrographs are presented showing an active process of neuronophagia in the cerebral cortex in mice starved to inanition, to illustrate the nature of the process, which is essentially a cytolytic one. Neuronophagia is also shown on slides of cerebral cortex from senile mice and from men 60 years of age and over. In these senile brains the process of neuronophagia, while not so widespread nor rapid as in starving animals, is of essentially the same nature.

Phagocytosis of one nerve cell in senile animals is more often accomplished by several glial cells, while in younger starved animals a single glial cell may often be observed to phagocytize an entire nerve cell.

The slides illustrate the fact that there is a very definite increase in the amount of satellitosis about the large pyramidal cells of the cerebral cortex with advancing age. Sections of the cerebellum, however, show that this is not true of the Purkinje cells, nor has any process of neuronophagia been observed in these cells either in the mouse or in man.

Age changes in the nerve cells of man and mouse include an increase in basophilic properties of the nucleus, a loss of Nissl material, a loss of regularity of cell outline, and, in some cases, the formation of pigment in the cytoplasm. (See abstract of paper.)

D3. Differential staining for the nuclei of the suprarenal cortical cells. Dan D.

Baker, Department of Anatomy, Louisiana State University Medical Center, New Orleans. (See abstract of paper.)

D4. Normal development of the lens in the albino rat and the effect of a diet high in galactose. Stephanie L. Bannon and Helen W. Kaan, Department of Zoology and Physiology, Wellesley College.

The lens appears as an epithelial thickening in embryos of 10 days' gestation. The vesicle is completely closed at 12 days and thickening of the posterior wall has commenced. At 15 days, vacuoles appear in the tissue at the posterior pole of the lens. They persist through the sixteenth day but disappear in lenses of 17-day embryos and are not present at birth. A female rat, fed throughout the

gestation period on a diet high in galactose, will give birth to young which show evidences of cataract. The lenses of such individuals contain vacuoles of varying sizes, occurring chiefly at the posterior pole of the lens nucleus.

D5. Normal and experimentally altered end bulbs in the cat's spinal cord.

Murray L. Barr, University of Western Ontario. (Introduced by C. C. Macklin.)

Sections of cat's spinal cord prepared by modification 4 of Cajal's reduced silver nitrate method will be presented to illustrate the following observations:

1. Axon terminations on cells of the lateral groups of the ventral horn are smaller than those on cells of the medial groups of the ventral horn and the chief portion of the dorsal horn.

2. End bulbs are present in the region of the axon hillock and on the proximal portion of the axon as well as on the cell body and dendrites of neurons.

3. Axon terminations tend to become smaller on cells undergoing retrograde degeneration. In later stages many endings either atrophy completely or become refractory to the silver. Endings on dendrites may persist after most of those on the cell body of the degenerating neuron have disappeared. (See abstract of paper.)

D6. Materials for a history of human growth and developmental anatomy.

Lawrence B. Benson, Averill F. Little and Richard E. Scammon, The Graduate School, University of Minnesota.

No history of the study of human growth and developmental anatomy has yet appeared in print. The demonstration here presented is a sample of material illustrating such an account. It consists of portraits of the chief students of the field both in recent and more remote times, originals of some of the rarer and more interesting publications, examples of the pictorial, graphic and analytical methods used (usually the first in each instance), facsimilies of title pages and other portions of important works, and a synoptic table of the chronology of studies in this field.

D7. Elimination of particulate matter from the abdominal and thoracic cavities in the rat. Janet Bloch and Madeleine P. Grant, Sarah Lawrence College, Bronxville, New York.

Elimination of particulate matter injected into the peritoneal cavity takes place by two independent processes: 1) Absorption of granules into the lymphatic system, drainage through the diaphragmatic plexus, and phagocytosis of the granules by cells of the thoracic lymph glands. 2) Phagocytosis and storage of the granules by groups of cells in the great omentum.

Removal of the great omentum does not affect the drainage process through the lymphatic system. The presence of the omentum is not essential for elimination of foreign particles injected into the abdominal cavity, but when present it greatly aids in the elimination process.

Absorption of particulate material injected into the thoracic cavity takes place by two processes: 1) Absorption into the lymphatic system and phagocytosis of the granules by cells of the thoracic lymph glands. 2) Phagocytosis and storage of the granules of groups of cells in the mediastinum.

D8. A demonstration case of the skull for student study. Raymond F. Blount, Department of Anatomy, University of Minnesota.

Photographs are shown of a case for the demonstration of the skull bones in which each specimen is so mounted that it may be rotated by the student for complete viewing. The case itself is 6 feet long with a glass front and top each 1 foot wide. It is mounted above a table for books or notes. A bench is provided which may be turned back when not in use. The rotation of specimens is mechanically accomplished by having the supporting rods extend through the floor of the case and these are provided with knobs for turning. There are twenty-eight mounts which can be completely revolved without collision and some space remains for additions. The exhibit contains all the individual skull bones mounted in various orientations together with cut and dissected skulls.

D9. Analysis of growth in crown-heel and crown-rump length from the second month of prenatal to the third year of postnatal life. Edith Boyd, Department of Anatomy, University of Minnesota.

The central trend of growth from the second fetal month to the third year of postnatal life in both crown-heel and crown-rump length with menstrual age may be adequately represented by relatively simple exponential equations. The differences between equations fitted to data collected by Mall before 1910 and those fitted to data collected since 1920 appear to be within the error of sampling. The first derivatives of the equations show that the absolute rate of growth of each dimension reaches the maximum at about 100 days of menstrual age. The relative first derivatives are hyperbolas which show that the relative rate of growth decreases throughout the fetal period.

The probable error of individual cases in the fetal period around the equations fitted to modern data is 2.6 cm. for crown-heel length and 2.1 cm. for crown-rump length. The five mnemonics and nine curves or equations proposed during the past century for computing fetal age from length for all or part of the prenatal period fall within plus and minus two probable errors of the fetal portion of the circumnatal trend fitted to modern data, with a preponderance of curves between the trend and minus one probable error. Of all the mnemonics and curves proposed, that of Quetelet for the last 5 fetal months most closely approaches the curve based on modern data.

D10. The effects of crystalline sex hormones upon sex differentiation in the pouch young of the opossum. Robert K. Burns, Jr., Department of Anatomy, The University of Rochester, School of Medicine and Dentistry.

The young of the American opossum (*Didelphys virginiana*) are very immature at birth. Sex differentiation is beginning in the gonads but dimorphism in the other parts of the reproductive apparatus does not occur until much later. Treatment with crystalline sex hormones during the first weeks of pouch life produces striking effects upon the differentiating parts of the reproductive system.

Testosterone propionate produces enormous hypertrophy of the phallus in both sexes, with quantitatively comparable results. Scrotum and marsupium are apparently unaffected. Wolffian ducts differentiate precociously into vasa deferentia and epididymi in both sexes, and rete connections are present. Prostates hypertrophy and bulbo-urethral glands proliferate. However this hormone likewise induces development of all mullerian duct derivatives, especially in females, uterine development in males is marked and sufficient to arrest descent of the testes, but vaginae are usually absent. These effects are prominent at 3 weeks and extreme by the seventh week of pouch life.

Estrone also produces hypertrophy of the phallus, but of a different and characteristic type. Marsupium and scrotum are not definitely modified. Internally there is specific stimulation of mullerian duct derivatives; male parts are unaffected.

It appears that estrone (on the basis of more limited material) is specific for female structures only; testosterone propionate, however, exerts a dual action on both male and female parts.

D11. I. Microscopic preparations and photomicrographs of contraction compression waves in smooth, transitional and striated muscle. Eben J. Carey, Department of Anatomy, Marquette University School of Medicine.

II. Distribution of mineral ash in contraction compression waves in smooth, transitional and striated muscle. Eben J. Carey and Walter Zeit, Marquette University School of Medicine.

This demonstration will consist of microincinerated preparations by Scott's method and photomicrographs on the variable distribution of inorganic salts in the contraction compression waves of active and relatively inactive smooth, transitional and striped muscle. In smooth intestinal, transitional gizzard and striped voluntary muscles the nodes have increase of inorganic salts, whereas, the internodes have a decrease. The contraction compression wave in smooth, transitional and striped muscle is identified in alternate serial sections of ordinary microscopic and microincinerated preparations as consisting of two parts: 1) the transverse pressure node and 2) the longitudinal tension internode. Phase differences in both the pressure and tension components of longitudinal compression muscle waves will be demonstrated by the variable distribution of the mineral ash. There is a progressive shortening in the wave length and increase in number of pressure waves revealed by mineral ash in active smooth, transitional and striped muscles. The mineral skeleton of fixed muscle waves varies either in amplitude by increase of mineral ash condensation or in frequency by increase in number of the transverse pressure bands of inorganic salts. The experimental histologic evidence from these three types of muscle warrants the conclusion that the underlying compression wave mechanism of action of these muscles is the same and that the fundamental difference is quantitative and not qualitative.

D12. Observations on the nuclei of stromal cells in human endometrium. Rucker Cleveland, Department of Anatomy, Vanderbilt University School of Medicine. (See abstract of paper.)

D13. The development of the neural crest. J. LeRoy Conel, Department of Anatomy, Boston University School of Medicine and Massachusetts Memorial Hospitals.

The slides and photographs show various stages in the development of the neural crest in *Bdellostoma stouti*, *Squalus acanthias*, *Torpedo* and chick. The entire crest arises in the same manner as the optic vesicle, i.e., as an evagination of the dorsal part of the alar plate. Development proceeds in a cephalocaudal direction.

D14. Demonstration of a connective tissue layer in the adult which is the fascia of fusion of Toldt expanded to keep pace with the growth of the body and not greatly modified from its original form. Edgar D. Congdon, Department of Anatomy, Long Island College of Medicine.

This connective tissue layer on the left side of the body extends from the anterior surface of the suprarenal gland or the right crus of the diaphragm to the attachment of the pelvic colon at the pelvic brim. On the right side it usually reaches from the attachment of the transverse mesocolon to the peritoneal reflection which bends from the parietes onto the caecum. The fascia on the left side terminates laterally by going over onto the connective tissue layer of the peritoneum either where it passes from the parietes to the wall of the omental bursa, or where it passes from the parietes to the colon. On the right side its lateral boundary is marked by fusion with the peritoneum of the paracolic gutter.

Medially the right and left fasciae terminate rather variably near the midline.

The fasciae of the two sides taken together constitute the chief topographical boundary plane of the abdominal cavity other than the peritoneal cavity itself. (See abstract of paper.)

D15. A teaching model to illustrate the development of the omental bursa. Edgar D. Congdon and George H. Paff, Department of Anatomy, Long Island College of Medicine.

It is admitted that the use of a wood and cloth model to demonstrate a growth problem involving differential rates invites misinterpretations. However, the development of the omental bursa is so difficult for the average student to grasp that the return justifies the risk. For several years we have used such a model with the result that students are better prepared to understand the gross anatomy of this region. In a few minutes the student is able to apply visually his textbook description by actually rotating the stomach and watching the bursa develop. In addition, the hair-pin loop of gut can be rotated to simulate reasonably well the fixation of the large and small intestine.

D16. The occurrence and distribution of a modified acidophile cell in the anterior pituitary of the female monkey, Rhesus macacus. Alden B. Dawson, Biological Laboratories, Harvard University.

A modified acidophile cell has been demonstrated in the anterior pituitary of the monkey. This cell is characterized by its coarse granulation, large Golgi

apparatus and affinity for azocarmine rather than orange G, following fixation in formalin-corrosive sublimate and staining with Heidenhain's azocarmine, orange G—anilin blue. It appears comparable to similarly stained cells demonstrated in the female rabbit, cat and ferret. Eighteen pituitaries, obtained through the courtesy of Professor Hisaw, were taken from normal and castrate females, following various treatments with estradiol, progesterone and pregnancy urine. The carmine cells vary greatly in size, number and distribution, being fewest in castrates after a light treatment with estradiol and reaching an apparent maximum after prolonged treatment with a combination of estradiol and progesterone. When these cells are few in number they tend to be in a localized anteroventral zone clearly marked off from the rest of the anterior lobe by the restricted occurrence of orange acidophiles and predominance of basophiles or chromophobes. In the rabbit and cat this region has been designated as the zona tuberalis of the anterior pituitary on account of its gradual transition dorsally into typical tuberalis tissue. When the carmine cells are more numerous they also appear massed in the regions adjacent to the zona tuberalis but may, in extreme cases, occur diffusely throughout the entire anterior lobe.

D17. Experimental production of a leukemoid state. Tom Dougherty, Department of Pathology, The University of Oklahoma School of Medicine. (Introduced by Berry Campbell.) (See abstract of paper.)

D18. Blood pressure studies on rabbits. F. W. Dunihue, Department of Anatomy, University of Vermont Medical College. (Introduced by O. M. Helff.)

While studying the effect of aspartic acid on the blood pressure of rabbits, it was noted that 'normal' animals are subject to sporadic, temporary elevations of blood pressure which seem to be correlated with attacks of spontaneous nephritis. Experimental rabbits receiving intramuscular injections of neutralized aspartic acid exhibited more persistent, mildly hypertensive states which were accompanied by intensive attacks of spontaneous nephritis and toxic injury to the tubular epithelium. It is suggested that aspartic acid is not an hypertensive agent per se, and that the longer duration of the moderate hypertension in the experimental animals is due to the nephrotoxic action of the acid increasing the susceptibility of the kidneys to attacks of spontaneous nephritis.

Blood pressure charts and histological preparations of kidneys from 'normal' and experimental rabbits will be demonstrated.

D19. Structure of the ellipsoids in various species of vertebrates. P. Dustin, Jr., Harvard University and University of Brussels, Belgium. (Introduced by F. T. Lewis.) (See abstract of paper.)

D20. The pigment forming capacity of the blastoderm of Barred Plymouth Rock embryos as shown by transplants to White Leghorn hosts. Herbert L. Eastlick, Department of Zoology, University of Missouri. (Introduced by Mary J. Guthrie.)

A study of the source of melanophores in the fowl has been made by grafting small pieces of early blastoderm of Barred Plymouth Rock embryos to the lateral

body wall or into the intra-embryonic coelom of White Leghorn hosts. The donor tissue was secured from unincubated eggs and succeeding stages through the head fold. The hosts possessed between 26 and 35 somites.

So far, pieces of the blastoderm of all stages preceding the definitive primitive streak have failed to produce pigment. At the primitive streak stage, however, a piece of blastoderm 0.2 mm. or more in diameter, which includes any portion of the node, usually produces a patch of colored feathers when grafted to the lateral body wall. Moreover, the nodules which are formed when the grafts are made into the coelom ordinarily possess colored feathers. In all cases so far obtained (five), pieces of blastoderm more than 0.3 mm. posterior to Hensen's node have failed to develop pigment. In head process and head fold stages, the area capable of producing pigment seems to extend forward and lateral to the node. The exact limits are not known at present.

These results seem to indicate that the pigment-forming area of the blastoderm corresponds quite closely to the region which is concerned with the formation of neural tissue.

D21. A section method for critical staining and differentiation of the unmyelinated fibers in peripheral nerves. James O. Foley, Departments of Anatomy, University of Alabama and Cornell Medical College.

Stretches of peripheral nerves are mordanted after fixation in a 2% aqueous solution of silver nitrate. Nerves which are fixed in acidified solutions of formaldehyde are rinsed in distilled water and put directly into silver nitrate. Those fixed in fluids containing salts of mercury or chromium are given a preliminary treatment with tincture of iodine before they are silvered. Following silvering in the block the nerves are, without reduction, dehydrated and embedded in paraffin. Thin sections of the mordanted nerves are then silvered and reduced on the slide by either the alcoholic silver nitrate method of Davenport or the activated protargol method of Bodian. Subsequently and in conjunction with gold toning the sheath matrix, in which the unmyelinated fibers are embedded, is counterstained with aniline dyes. Critical staining of Schwann's sheath substance in colors which contrast with the black nerve fibers makes possible a more positive identification of individual axons. The unmyelinated character of the sympathetic trunk of the cat, dog and rat will be demonstrated in sections prepared with the technique.

D22. Foetal anomalies. A. W. Fuchs, Eastman Kodak Company, Rochester, New York. (Introduced by W. E. Sullivan.) (No abstract.)

D23. Observations on the so-called hibernating tissue in the white rat. Madeleine P. Grant, Sarah Lawrence College, Bronxville, N. Y.

For 3 years a small group of students have been studying the so-called hibernating tissue in the white rat. Meredith Shelton Carson was the first to stimulate interest in the tissue. Anatomical illustrations showing locations of the tissue throughout the body have been made by Elinor Sewall. The effect of the

removal of the 'dorsal butterfly' portion was studied by Jane Phillips. Preliminary observations on the effect of adrenalectomy on the tissue have been made by Jane Phillips and Virginia Hewitt. Drawings, microscopic slides and graphs prepared by students will be demonstrated.

D24. Effect of testosterone, progesterone or both on the monkey uterus: Administration by injection or by means of Deansley-Parkes pellets. Carl G. Hartman, Department of Embryology, Carnegie Institution of Washington.

In one series of twelve mature castrated rhesus monkeys, six received hormones hypodermically in oil (group I), six received pellets of pure crystals after the method of Deansley and Parkes (group II). All were primed for 14 days with oestrone (Amnotin Squibb in oil, pellets of Progynon Schering or Theelin Parke-Davis). Two animals in each group then received oestrone plus progesterone (Proluton Schering), two oestrone plus testosterone (Testosterone propionate Ciba), two all three hormones; those in group I were exposed to the hormones for 19 days, those in group II for 14 days. Pellets proved almost as effective as material injected in oil. Ten milligrams daily of testosterone failed completely to mimic the progestational effect of 1 mg. progesterone. Administered concomitantly with progesterone the androgen had no inhibitory effect but possibly an additive one, as in no. 540 which furnished the 'best' endometrium of all three hormones, injected. No. 576 had a pregravid endometrium 5 mm. in thickness on day 29 of an ovulatory cycle in spite of receiving injections of 25 mg. testosterone propionate daily on days 21 to 27 inclusive. Other cases, with biopsy controls, confirmed these conclusions. In no. 330 10 mg. daily of testosterone (without oestrone) maintained a fairly proliferative endometrium for 60 days.

Microscopic preparations, together with photographs and the appropriate protocols, will be demonstrated.

D25. On a complete normal 12-day human ovum of the pre-villous stage. Arthur T. Hertwig and John C. Rock, Free Hospital for Women, Brookline, Massachusetts; Departments of Gynecology, Obstetrics and Pathology, Harvard Medical School, Boston; and Department of Embryology, Carnegie Institution of Washington. (Introduced by Dr. George L. Streeter.) (See abstract of paper.)

D26. Sections of tissues prepared by the Altmann frozen dehydration method. Normand L. Hoerr, Department of Anatomy, The University of Chicago.

The advantages of the Altmann method for the study of cytoplasmic structures such as mitochondria, Golgi apparatus, and liposomes are presented. The limitations of the method for histochemical studies are demonstrated. The method can be used to avoid the post mortem and fixation artefacts obtained in other methods of microscopic preparation, in some organs such as spleen, stomach and adrenal.

D27. The use of rubber (latex) in the making of flexible molds for reproducing anatomical specimens. Karl F. Hoffman and Gustave J. Noback, Departments of Anatomy, College of Dentistry and the Graduate School, New York University.

Many of the difficulties in the making of molds from which more than one cast of a specimen may be made are eliminated by the use of rubber (latex).

The process of making a latex mold will be demonstrated by means of a series of preparations showing each stage in the evolution of the mold. Casts in several different materials made from the mold will also be shown and photographs of the operations involved will be on view.

D28. The afferent nature of the innervation of the glomi aortici. W. H. Hollinshead, Department of Anatomy, Duke University School of Medicine.

The epithelioid bodies described by Nonidez under the name *glomi aortici* receive a rich innervation through the vagus nerve, as Nonidez has already shown. In the present experiments on cats, the nerve fibers among the cells of the right glomus have been found to disappear following section of the right vagus nerve below the ganglion nodosum, but have remained intact following removal of the superior cervical ganglion on that side. The innervation is thus through vagal fibers, rather than through sympathetic fibers accompanying the vagus. Moreover, the fibers have also been found apparently intact following section of the vagus above the ganglion nodosum, indicating that the cells of origin of these fibers are located in this ganglion. Since the available evidence concerning the efferent fibers supposed to arise from cells in the ganglion nodosum indicates that these are among the finer fibers in the vagus, the larger fibers reaching the *glomi aortici* can hardly be of this nature, but would seem to be true sensory fibers. These experiments, therefore, have confirmed Nonidez's belief in the sensory nature of the *glomi aortici*, and have reinforced the comparisons which he has drawn between these bodies and the carotid glomi.

D29. A comparison of the tissue components of newborn and adult extremities. Jacob Jacoby, Department of Anatomy, University of Minnesota. (Introduced by Edith Boyd.)

Cross sections were made of the arm, forearm, thigh and leg of a formalin-fixed full-term female newborn. The outlines of the tissues were traced on ground glass. The tracings were enlarged by means of a photographic projection apparatus to be of equal cross sectional area with comparable sections of adult extremities (from Eichelsheimer's 'Cross Section Anatomy'). By this method the enlargement of each element is proportional to that of the total. Drawings are presented of the actual and projected tracings.

Cross sectional areas of skin, muscle and bone were obtained with a planimeter. The area of fat and connective tissue was obtained by subtraction of the sum of the measured parts from the total.

Fat decreased from about 50% of the total area in the newborn to about 30% in the adult. Muscle increased from about 35% in the newborn to about 55% in the adult. These changes were more marked in the upper extremity than in the lower. Skin showed a relative decrease and bone an increase in amount from newborn to adult. A comparison is shown in the bar diagram.

D30. Studies on the origin of sympathetic ganglia and of sheath cells in the chick embryo. David S. Jones, Department of Anatomy, Loyola University, Chicago.

Progressive stages in the development of sympathetic ganglia and sheath cells are shown in a series of normal embryos. Stages in the development of these cells are also followed in a series of embryos from which portions of the neural crest were removed at about 45 to 48 hours of incubation. Comparison of the two series shows that the formation of the sympathetic ganglia and of the sheath cells on the ventral root fibers is very much the same in the two, except for the following differences: In the experimental series the migration of cells from the neural tube along the ventral root fibers is delayed; the ventral root fibers remain naked until cells from the neural tube migrate into the roots and differentiate into neurilemma cells; the sympathetic ganglia are about 1 day late in making their appearance, but develop as usual from cells which migrate from the neural tube along the ventral root fibers.

D31. Effect of antianemic principle on the primitive erythroblasts of the 11-day rat embryo. Oliver P. Jones, Department of Anatomy, University of Buffalo Medical School.

The first three demonstrations are dry smears of blood from the normal rat yolk sac of 11-, 12- and 13-day embryos. These preparations were made according to the method described by Kirschbaum (Toronto meetings). The primitive erythroblasts in these preparations serve as controls for the fourth and fifth demonstrations which show the effect of antianemic principle on these cells. The sixth demonstration is a dry smear of normal rat bone marrow stained with May-Grünwald-Giemsa combination of Pappenheim. Compare these cells of the normoblastic or definitive red blood cell series with the primitive erythroblasts of the prehepatic period. Note that a superabundance of antianemic principle did not transform the primitive erythroblasts into cells of the definitive generation. (See abstract of paper.)

D32. Apparatus and dissected human foot showing how much support the arch receives from the long muscles of the foot. Russell L. Jones, Department of Anatomy, Indiana University School of Medicine.

By the method reported at the Anatomists' meeting in 1938 additional data has been secured which indicates the following.

The stress on the human longitudinal arch varies directly with the pressure at the ball of the foot and not with the weight on the tibia. The posterior tibial muscles and the peroneus longus contribute from 15 to 20% to the support of the arch. The plantar ligaments, muscles and fascia contribute the balance. The anterior tibial contributes nothing, functioning chiefly as a dorsiflexor.

The most significant effect of certain long leg muscles is the shift of pressure from the lateral four metatarsal heads to the first and vice versa. This shift of pressure or weight-bearing capacity occurs without noticeable inversion or eversion. The muscles differ in their efficiency in producing this shift. A given tension on the peroneus longus effects a shift to the first metatarsal four times as

great as the shift to the lateral four metatarsals produced by the same tension on either the posterior tibial or the flexor digit. longus. The flexor halluc. longus produces no appreciable shift.

This active muscular shifting of the pressure preserves the optimal functional distribution of pressure on the metatarsal heads by compensating for intrinsic and extrinsic factors which alter that distribution.

D33. Two similar cases of an anomalous lobe of lung tissue not attached to the respiratory system, together with a proposed classification of anomalies of the respiratory system. Hovey Jordan, College of Medicine, University of Vermont. (Introduced by G. H. Parker.)

Two similar cases of an anomalous lobe of somewhat atypical lung tissue were found at successive autopsies. In each case this lobe was not connected to any part of the essentially normal respiratory system; but, rather, was covered by a reflection of the parietal pleura which, also, formed a pedicle serving to attach the anomaly to the posterior body wall just lateral to the spine and slightly above the diaphragm. In one case this anomalous lobe had an independent and anomalous blood supply. The probable embryonic source is thought to be an evagination from the embryonic gut caudal to and separate from the respiratory anlage in each case. This conclusion is based, primarily, upon a consideration of the level of attachment of the pedicle, the morphology of the mediastinum and related structures during development and upon the independent blood supply. A suggested classification of anomalies of the respiratory system is also given. The anomalies will be demonstrated by means of photographs, histological slides and drawings. One of the anomalies will be shown. The proposed classification of anomalies of the respiratory system will be demonstrated by means of charts and tables.

D34. Improved hollow-tipped fused quartz living tissue illuminator. Melvin H. Knisely, Department of Anatomy, The University of Chicago.

Dm56. Microscopic observations of the circulatory conditions in living frog liver lobules. Melvin H. Knisely, Department of Anatomy, The University of Chicago.

Living untraumatized livers of pithed or urethanized frogs studied at 96 to 600 $\times$ show that the lobule's vessels control 1) the environment of hepatic cells, varying it to an apparently significant degree, 2) the storage and release of whole blood or red cells by sinuses, thus regulating the circulating blood volume and peripheral red cell count, and 3) the time and rate at which the liver yields lymph.

Afferent hepatic arterioles and portal venules are both sharply contractile. With all arterial tips shut sinusoids receive portal blood.¹ With portal tips shut and arterial open, sinusoids receive pure arterial blood. The tonus of and pressures in the two afferents control the proportions of mixed arterial and portal blood sinuses receive.¹

Linings of sinuses receiving mixed or portal blood exhibit two (perhaps three) permeability phases, one, as impermeable to blood colloids as ordinary endothelium,¹ the other nearly or completely permeable to blood plasma.¹ During one the lobule yields no lymph. During the other lymph production is proportional to blood flow.

A sphincter guards each sinus's exit.¹ They act individually,¹ in groups¹ or all together.¹ Colloid impermeable walls plus closed sphincters yield storage of whole blood.¹ Plasma permeable linings plus closed sphincters yield blood cell storage.¹ Opening of sphincters releases blood or cells to veins.¹

¹ Cinema recorded.

D35. The lymphocyte in acute inflammation. Fred Kolouch, Jr., Department of Anatomy, University of Minnesota. (Introduced by Hal Downey.) (See abstract of paper.)

D36. Models of the Minot and Herzog human embryos. Frederic T. Lewis, Department of Anatomy, Harvard Medical School. (See abstract of paper.)

Dm57. An animated motion picture of the development of the human nervous system. Joseph J. McDonald, Department of Anatomy, Northwestern University Medical School, Chicago, Illinois. (Introduced by L. B. Arey.)

The demonstration includes some representative portions of a teaching film designed to show the development of the nervous system. The following growth sequences will be illustrated: development of body form from the presomite embryo to the 10-mm. stage; formation of the neural plate, neural groove and neural tube; appearance of the neural crests and development of spinal ganglia, including the histogenesis of ganglion cells; peripheral growth and myelination of nerve fibers; histogenesis of neurons in the spinal cord; establishment of motor endings on muscle and sensory endings in the skin; development of the sympathetic system; and the recession of the spinal cord in the vertebral canal during fetal growth.

D37. The structure and topography of the various joints of the body in infancy, childhood and adolescence. The late Lloyd E. McFarlane and Richard E. Scammon, The Graduate School, University of Minnesota.

Although the development of joints has been studied by radiography in detail in recent years, there have been almost no additions to our knowledge of their structure or topography between birth and maturity by the use of dissections or section methods. This seems due to the great difficulty in securing material. This demonstration presents drawings of three sets of sections of developing joints in postnatal life (including the soft tissues associated with them).

1. Eight sections of joints of a child 1 year of age (after Corning).
2. Twenty sections of joints of individuals ranging in age from birth to 19 years (after von Brunn).
3. Forty sections of joints of individuals ranging in age from birth to 2 years (original series).

D38. Sections showing a continuous layer of cuboidal epithelium on the alveolar walls of the cat's lung after exposure to the fumes of osmium tetroxide. Charles C. Macklin, University of Western Ontario.

Air which had bubbled through a 1% solution of osmic acid in water was insufflated via an urethral catheter into the lungs of anesthetized cats for $\frac{1}{2}$ minute: and sections were taken at varying stages thereafter. On the fourth day there was a marked hypertrophy and multiplication of the epithelium. In places a continuous layer of cells appeared, lining the alveoli. These cells were irregular in size and shape, resembled those of the bronchiolar epithelium, and sometimes formed nodular masses. These epithelial layers resembled those often described in human lungs from patients who had been exposed to irritating fumes, such as those of nitric acid: a condition which has been termed 'chemical pneumonia,' and has proved fatal. They doubtless interfere with gaseous exchange. They suggest the continuous epithelial layers on the alveolar walls of the early embryo: but the view is dissented from that they necessarily support the theory of a continuous layer of epithelium on the mature mammalian alveolar wall. Rather the interpretation is suggested that we are here dealing with an hypertrophy and proliferation of the scattered cells of entodermal origin, known as epicytes, which are normal constituents of the alveolar walls of the mature mammal. Sections showing such continuous epithelial layers will be shown.

Dm58. Movements of the shoulder joint. C. P. Martin, Department of Anatomy, McGill University, Montreal, Canada.

The film demonstrates the fact that, when the arm is elevated in the coronal plane, the humerus must rotate laterally, and endeavors to answer the problem of the mechanism which brings about this lateral rotation.

D39. Physiology of the dimorphism in the female honeybee (Apis Mellifica L.). R. M. Melampy, U. S. Department of Agriculture and Department of Zoology, Louisiana State University. (Introduced by B. I. Burns.)

Charts, curves photographs, slides, and specimens will be used to illustrate the development of the reproductive and worker castes of the female honeybee (*Apis mellifica* L.). The two castes appear to be thresholds of development due to a difference in food. A reduction in quantity of food intake produces dwarf forms but it is doubtful if caste production can be explained on this basis. The reproductive caste receives a diet which is secreted by the pharyngeal glands of the worker caste. The secretion has the following chemical composition: water 66%, dry matter 34%, protein 12%, total reducing substances 12.50%, lipid 5.00%, and ash 0.80%. This material contains little, if any, vitamins A, C or E, whereas it is a good source of vitamin B. The protein has a biological value of 75% and a digestibility coefficient of 95%.

D40. The source of the nerve supply to the pancreas of the cat. Russell L. Moseley, Departments of Anatomy, University of Minnesota and West Virginia University. (Introduced by Dr. A. T. Rasmussen.)

It has been established by several investigators that, in addition to nerves to pacinian corpuscles, in the cat's pancreas, there are autonomic ganglion cells, and single nerve fibers ending in the walls of arterioles, ducts, and about the islands of Langerhans. To determine the origin of these nerves fibers, cats were subjected either to bilateral vagotomy (below the diaphragm), or to bilateral splanchnic neurectomy, or to removal of the celiac ganglia, and allowed to live various periods of time from 1 to 12 days after operation.

Because of the relative scarcity of pericellular fibers in the pancreatic ganglia, their disappearance after operation was not the sole criterion used to indicate degeneration. On the other hand, positive early changes of the nature of swelling and fragmentation have been sought for in the pericellular fibers in Cajal and Bodian preparations. However, unequivocal changes in intraganglionic nerve fibers can, at present, be reported after neither vagus nor splanchnic section, but only after removal of the celiac ganglia. Likewise, degenerative changes in the nerves to the islands of Langerhans, the arterioles, and duct walls have not been seen after vagus or splanchnic section. Degeneration of the central nerve fibers in the pacinian corpuscles has been identified 3 days after splanchnic section. These results confirm part of the conclusions reached after similar experimental work by Kubo and Miyagawa.

Dm59. Motion pictures of fetal movements in the rabbit. D. S. Pankratz, College of Medicine, University of Tennessee.

In 15-day-old fetuses no spontaneous or elicited movements are seen. Sixteen-day-old fetuses show lateral upper trunk flexion, the forelimb moving with the trunk. Sixteen and one-half to 17-day-old fetuses show movement of the forelimb and lateral trunk flexion on stimulation of forelimb or head, especially the snout. Eighteen-day-old fetuses show gasping-like movements, simple slow hind limb movements, alternating forelimb movements, and opening and closing of mouth. In 19- to 29-day-old fetuses there is independent hind limb movement and the forelimbs move to points stimulated on the face. At this age there is flexion at the carpal joints and spreading of the toes in the forefoot. Twenty-two-day-old fetuses show the righting reflex and crossed reflexes in the forelimbs. In 23-day-old fetuses there is movement of the tail, crossed hind limb reflexes and withdrawal of the limbs. Stimulation of the snout causes head extension, opening and closing of the mouth accompanied by tongue movement. Sucking and active righting reflexes are present in 25- to 26-day-old fetuses. All movements elicited by stimulation also appeared spontaneously. From the twenty-sixth day to birth all movements become quicker and more complete.

D41. The culture of human ovarian ova. Gregory Pineus, The Biological Laboratories, Clark University. (Introduced by G. B. Wislocki.)

A demonstration of the methods of obtaining and culturing human ovarian ova and a presentation of ovum preparations obtained under various experimental conditions.

D42. Effects on embryos of x-radiation of frog spermatozoa. Roberts Rugh, Department of Zoology, Columbia University. (See abstract of paper.)

D43. A demonstration of nerve fibers in the metaplastic epithelium of vitamin A deficiency. Mary Elmore Sauer, Department of Histology and Embryology, University of Texas. (Introduced by Donald Duncan.)

The transformation of many simple epithelia into the stratified, cornified variety is a characteristic sequel of vitamin A deficiency. Despite extensive study of the nervous system in this condition, no observations have been reported upon the innervation of the metaplastic epithelium. In order to investigate this question, Rogers' stain was applied to tissues of vitamin A deficient rats. This technique revealed structures within the metaplastic layer which appear to be nerve endings. These can be followed into the epithelium from below and seem to end upon the epithelial cells. Since the normal epithelium degenerates, the nerve fibers in the metaplastic layer must be new growths rather than a chance retention of the original epithelium of the affected region. Although nerves are present at all stages of metaplasia, they are more abundant in the older growths. The number of fibers present is not great, a finding which is analogous to that of others for unspecialized stratified cornified epithelium.

In the light of these findings, Mellanby's hypothesis of loss of neurotrophic control is not tenable for the origin of the metaplasia.

D44. A genetic approach to the interpretation of anatomical anomalies. P. B. Swain, E. L. Green, T. E. Tetrault, Brown University, and H. W. Edmonds, Washington University. (Introduced by William C. Young.)

Previously reported investigations of skeletal and vascular variations in the domestic rabbit concerned the number of ribs, presacral vertebrae, ventral spinous processes, and sternbrae, and variations of the vessels arising from the aortic arch. This study is now expanded to include the branching of the large vessels of the neck and lumbar regions. Charts, x-ray films, and transparencies will be used to show the distribution of all these variations in five unrelated families, four of which are moderately inbred. Each family possesses not only characteristic body size and conformation, but also characteristic internal patterns of variation. The same is true for two subsequent generations of three different crosses between them, two of which have not been previously reported. Although the manner of transmission supports belief in the inheritable nature of each variation, in no case is a simple mendelian interpretation satisfactory and the patterns indicate neither complete genetic association nor complete independence.

Since most internal anomalies find their way into the dissecting room by chance, their antecedents are not known and their interpretation is theoretical at best. It therefore seems desirable to call attention to the importance and promise of this morphogenetic approach; first, to the problems of comparative anatomy, and second, to the problem of the relative importance of general and specific regional growth factors in body development.

D45. Quantitative evaluation of appositional growth of the hard tissues by vital staining and applications in experimental studies. I. Schour, M. M. Hoffman and M. C. Smith, Department of Histology, College of Dentistry, University of Illinois, and Department of Nutrition, College of Agriculture, University of Arizona.

Demonstration of models, colored photomicrographs and ground sections. Steps in the preparation of ground sections.

Intraperitoneal injections of a 2% solution of alizarine red S at a dosage of 100 mg. per kilogram produce chronologic landmarks in the form of sharp red lines which can be readily seen in ground sections in the dentin, cementum and bone calcifying at the time of injection. The distance between two successive injection effects divided by the intervening number of days will give the daily rate of apposition.

This method has been used in the analysis of the normal growth pattern of the hard tissues of the rat incisor and rat molar and in the study of alterations in growth produced by vitamin A deficiency. The rate of dentin growth in the incisor is characteristically altered in proportion to the severity and duration of vitamin A deficiency. The rate is accelerated in the enamel-covered dentin and decelerated in the cementum-covered dentin.

D46. Further studies on the resorption of deciduous teeth. H. H. Shapiro and W. M. Rogers, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Developing permanent tooth germs are usually considered responsible for shedding of the deciduous dentition. Since the root of a temporary tooth in man is sometimes resorbed when the permanent germ is congenitally absent, doubt is cast on the validity of this accepted theory of root resorption.

We tested this theory experimentally in kittens. The permanent canine tooth germ on one side of the mandible was removed without injury to the growing root of the temporary tooth. The opposite side served as the control.

The deciduous canine on the operated as well as on the unoperated side, was shed, indicating that the developing permanent germ is not the causative agent in the shedding of the temporary teeth.

We reported previously the results of a series of roentgen studies upon these experimental animals.

The microscopic findings verify and supplement the x-ray interpretation. The mechanism of resorption is similar on both operated and control sides. Normally in deciduous roots the outline of the area of resorption conforms to the general contour of the developing crown of the permanent tooth. In the absence of the tooth germ a similar contour is usually observed.

In our experimental animals osteoclasts and odontoclasts cover the surface where resorption is occurring. However some deviations from the usual histological picture of the process of root resorption were observed.

Dm60. Some phenomena of muscle contraction. Carl Caskey Speidel, University of Virginia. (1 reel; 15 min.)

These cine-photomicrographs reveal some of the histological changes in striated fibers of cardiac and skeletal muscle during various kinds of contraction. The pictures include muscle fibers of both vertebrate and invertebrate animals.

D47. Action of esteronc on the sex organs of immature male cats. William F. Starkey, Department of Biology, University of Pittsburgh. (Introduced by John C. Donaldson.) (See abstract of paper.)

D48. A new profile reconstruction of the Miller ovum. George L. Streeter, Department of Embryology, Carnegie Institution of Washington.

The new human ovum elsewhere described by Hertig has made it necessary to restudy the Miller ovum which is of about the same stage of development. By replotting its five available sections, the Miller ovum can be brought into harmony with the Hertig ovum, both as to size and as to its detailed form. The human ovum is much like the macaque in its development, differing principally in a more precocious and more active differentiation of primitive reticulum. It is concluded that in neither of these two ova has the definitive yolk sac yet appeared. The demonstration consists of the original reconstruction made in 1926 and a new one which is intended to supplant it.

D49. Anomalous epithelial structures in the myocardium of pig embryos. George F. Sykes, Department of Anatomy, Tufts College Medical School. (See abstract of paper.)

D50. An ovarian tumor in the rat accompanied by alterations of the external genitalia. C. Donnell Turner, Department of Zoology, Northwestern University.

Among a strain of inbred albino rats, occasional females were found which did not litter although caged indefinitely with males. These animals were observed to possess an abnormality of the external genitalia which is characterized by incomplete closure of the urethral folds and by a conspicuous clitoris. Seven such animals have been detected to date and laparotomies have disclosed invariably a tumorous involvement of both ovaries. The ovaries contain large cysts and show teratomatous changes. In one case, the tumor-like growth near the hilus of the ovary contained glands which resemble salivary glands. These glandular cells were arranged in acini and a distinct duct was present extending from the acini. The cystic cavities are lined by stratified squamous cells which appear to be teratoma-like. The cortical portion of the ovary contains a few moderately dilated follicles and many smaller ones in varying stages of atresia. Corpora lutea have not been observed. Extending into the stroma of the ovary are extensive fan-shaped areas of polyhedral cells. This appears to be a theca-cell tumor. These females remain in the diestrous state for long periods but occasionally give an estrous vaginal smear. Experiments to determine the hormone production of these pathological ovaries are in progress.

1951. *The masculinizing effect of some gonadotropic hormones on gallens compared with spontaneous ovariogenic tumours in hens.* Unto U. Uotila, Department of Anatomy, Harvard Medical School. (Introduced by George R. Wislocki.)

In immature pullets FSH stimulates the medullary cord cells, and, to a lesser degree, the interstitial cells of the ovary, resulting in masculinization of head furnishings. FSH also stimulates the interfollicular cells, resulting in hypertrophy of the oviducts. LH stimulates principally the interstitial cells of the ovarian medulla, producing masculinization of head furnishings. Pregnant mare serum hormone combines the effects of LH and FSH. Pregnancy urine hormone stimulates the interstitial cells very feebly. Androsterone causes only slight cortical atrophy and masculinization of head furnishings. Estrin inhibits comb growth to some degree and retards ovarian cortical development with atrophy of medullary components. Injections of FSH and LH produce masculinizing ovarian overgrowth which fulfill several of Meyer's morphological and physiological criteria for 'arrhenoblastomata.' A comparison is made between these experimental overgrowths and spontaneous ovarian tumors in hens, and marked similarities are noted. A working hypothesis concerning the genesis of masculinizing ovarian overgrowths is suggested. According to this hypothesis, FSH should be regarded as primarily responsible for overgrowths involving tubule and cord formation, while LH, by virtue of its action chiefly upon the interstitial cells, should stimulate the formation of less typical, solid 'mesenchymal' overgrowths. The results speak for a passive rather than an active participation of the adrenal glands in the experimental masculinization described here.

1952. *Permanent preparations of lamprey embryos preserved for paraffin sections after localized vital staining with Bismarck brown.* Richard Weissenberg, The Wistar Institute of Anatomy and Biology.

Sections of lamprey embryos (*Lampetra fluviatilis*) are demonstrated preserved after localized vital staining with Bismarck brown for the analysis of the localization of the presumptive organs (compare the papers of Weissenberg in *Sitzungsber. Ges. naturf. Freunde Berlin* 1932 and 1936, *Anatom. Anzeiger* Bd. 79 and 82). The Bismarck brown method developed by Weissenberg (*Arch. f. Entw. mech.* Bd. 118 and *Sitzungsber. Ges. naturf. Freunde Berlin*, 1929) gives the opportunity to transfer the vitally stained embryos through alcohol and cedar oil into paraffin and to study the localization of the stained areas in paraffin sections. The embryos are simply fixed in a mixture of 8.5 parts of absolute alcohol and 1.5 parts of cedar oil for 10 minutes, washed in absolute alcohol for a few minutes and transferred directly into cedar oil. They can remain there in cleared condition for years or can be embedded in paraffin at any time. The carriers of the localized vital staining in the lamprey embryos are chiefly the yolk granules. Some of the demonstrated preparations are now 10 years old. Without any loss of their brilliant color they still give a true representation of the stained areas of the living embryos.

D53. A comparison of the topography and composition of the body at birth and in the adult by some newer methods. Harry A. Wilmer and Richard E. Scammon, The Graduate School, University of Minnesota.

The changes in topography and composition of the body in postnatal life has been demonstrated by the following sets of pictograms.

1. Outlines of anterior, posterior and lateral views of the body of the newborn and of the adult drawn a) to the same amount of reduction, b) to the same standing height, and c) to the same area as presented in silhouette.

2. Pictograms of the skeleton of the body in situ of the newborn and of the adult as seen in anterior, posterior and lateral views drawn a) to the same amount of reduction, b) to the same standing height, and c) to the same silhouette area.

3. Pictograms of the musculature, fat and skin of the body of the newborn and of the adult as seen in anterior, posterior and lateral views drawn a) to the same amount of reduction, b) to the same standing height, and c) to the same silhouette area.

4. Pictograms of the viscera and central nervous system in situ of the body of the newborn and of the adult drawn a) to the same amount of reduction, b) to the same standing height, and c) to the same silhouette area.

The pictograms are based upon radiographs, dissections, casts and models made from cross sections and with the orthoscope.

D54. A radiological study of visceral and respiratory activities of the fetus in amnio. W. F. Windle, R. F. Becker, E. E. Barth and M. D. Schulz, Departments of Anatomy and Radiology, Northwestern University Medical School. (See abstract of paper.)

D55. Preparation of casts of the bony labyrinth. M. Wharton Young, Department of Anatomy, Howard Medical School.

The several steps involved in a simplified method for making casts of the internal ear are demonstrated in sequence. Disarticulation or removal of the temporal bone is unnecessary except for a small part of its petrous portion. Only the simplest apparatus and materials easily available to any anatomical laboratory are required for this procedure. Slight variations of the technique render it applicable to the casting of other body cavities. Specimens and models will be demonstrated.

This method has been reported in *Science*, vol. 86, no. 2244, p. 619.

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